THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR ...... Shin Sugiyama ......

TITLE OF THESIS ... Chiroptical Studies of Nucleosides and ...

Their Periodate Oxidized-Borohydride ...

Reduced Products ...

DEGREE FOR WHICH THIS THESIS WAS PRESENTED ... M.Sc. ...

YEAR THIS DEGREE GRANTED ...... 1982 ......

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

THE UNIVERSITY OF ALBERTA

CHIROPTICAL STUDIES OF NUCLEOSIDES AND THEIR PERIODATE OXIDIZED-BOROHYDRIDE REDUCED PRODUCTS

by

© SHIN SUGIYAMA

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND
RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE

IN

DEPARTMENT OF CHEMISTRY

EDMONTON, ALBERTA

SPRING, 1982

THE UNIVERSITY OF ALBERTA
FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled CHIROPTICAL STUDIES OF NUCLEOSIDES AND THEIR PERIODATE OXIDIZED-BOROHYDRIDE REDUCED PRODUCTS submitted by Shin Sugiyama in partial fulfilment of the requirements for the degree of Master of Science in Chemistry.

ABSTRACT

Fifty-four pentofuranosyl nucleosides and methyl β-D-ribofuranoside were oxidized with sodium periodate and subsequently reduced with sodium borohydride. After destroying the excess borohydride with aqueous phosphate buffer (pH 6.5), the resulting quantitated solutions were subjected to measurement of their long-wavelength optical rotations.

A previously noted empirical trend related nucleosides having β-D or α-L configurations with positive rotations for their oxidized-reduced products and nucleosides having α-D or β-L configurations with negative rotations for their enantiomeric oxidized-reduced derivatives. Most nucleosides in the present study gave results in harmony with this empirical prediction including adenosine$_{\text{ox-red}}$ derivatives with an additional asymmetric center. However, 6-azapyrimidine (1,2,4-triazine) and 6-azapyridine (pyridazine) nucleosides were exceptions with reversed rotations for their oxidized-reduced derivatives. This behavior is suggested to result from the intrinsic optical vector orientation in the chromophore rather than rotameric preferences.

C-nucleosides (pseudouridine and formycin) were methylated at the acidic ring nitrogen to circumvent degradation under the oxidation-reduction conditions. Paper electro-

phoresis using a borate buffer (pH 9.3) was found to be a useful analytical method in the present study. The precursor nucleosides and their oxidized-reduced products gave distinct well separated spots. The anodic migrations of β-D-pentofuranosyladenine nucleosides and adenosine$_{\text{ox-red}}$ are in the order: lyxo- $\geq$ ribo- > xylo- > ox-red > arabinofuranosyladenine.

Adenosine$_{\text{ox-red}}$ (43), tubercidin$_{\text{ox-red}}$ (209), inosine$_{\text{ox-red}}$ (135) and uridine$_{\text{ox-red}}$ (136) were isolated and subjected to solvent and pH effect studies. In aqueous solutions at different pH values, DMF and MeOH, rotation magnitudes were affected but drastic changes (e.g. reversal of the sign) were not observed. However, drastic changes in the rotations of adenosine$_{\text{ox-red}}$ (43), tubercidin$_{\text{ox-red}}$ (209) and inosine$_{\text{ox-red}}$ (135) were observed in pyridine, whereas uridine$_{\text{ox-red}}$ (136) behaved normally. This was attributed to the "stacking" effect with "aromatic" purine-type bases.

Qualitative rates of the reduction of periodate by nucleosides (determined spectrophotometrically at a periodate concentration of $\sim 10^{-7}$ M) were found to be: β-D-ribo- > α-D-ribo- > β-D-lyxo >> β-D-arabino- ≃ β-D-xylofuranosyladenine. After 1 min, several β-D-ribofuranosyl nucleosides reduced periodate 1.2-1.6 times faster than their corresponding α-D anomers. This difference is presumed to result from the increased steric hindrance

of the α structure. This presents a new method for assigning the configuration of a given anomeric pair of ribofuranosyl nucleosides.

ACKNOWLEDGMENTS

I would like to thank Dr. Robins for his advice and encouragement, Debbie and the children for their patience and lonely evenings, and all my family for their support.

TABLE OF CONTENTS

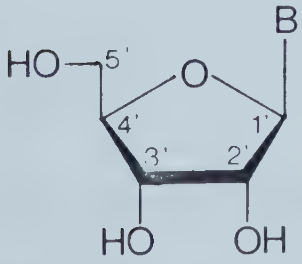
LIST OF TABLES

LIST OF SCHEMES

INTRODUCTION

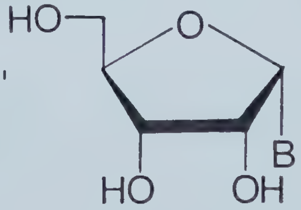
The term "nucleoside" was originally introduced by Levene and Jacobs[1] for the carbohydrate derivatives of purines (and later, of pyrimidines) isolated from the alkaline hydrolyzates of yeast nucleic acid. The phosphate esters of nucleosides are called nucleotides. "Nucleosides" now refer to carbohydrate derivatives linked to heterocycle bases, normally at C-1' of the sugar residue (anomeric center), by C-N or C-C bonds. The term includes both naturally occurring and synthetic compounds.

Since there are two possible orientations for attachment of the base at C-1' (namely, α or β as indicated for 1 or 2 in the D-ribose derivatives), the anomeric configuration determination of nucleosides has been a problem of considerable importance. This is especially true in synthetic nucleoside chemistry where coupling of an appropriate sugar and heterocycle (or its precursor) is frequently employed. Although the anomeric configuration of nucleosides found in DNA and RNA are known to



1

β at C-1'



2

α at C-1'

B = a heterocycle
or its precursor

be β, naturally occurring nucleosides having the α-configuration are also known in vitamin B_{12} coenzymes.[2] Thus, unknown naturally occurring as well as synthetic nucleosides always have to be subjected to determination of anomeric configuration.

A number of methods for determining the anomeric configuration of nucleosides have been devised. However, most of these, although useful in specific cases, have limitations and/or exceptions. Thus, the combined use of more than one technique is often required. X-ray diffraction provided the first confirmed structural assignments of certain nucleosides obtained from yeast nucleic acid.[3] This method gives a rigorous and unambiguous assignment of the anomeric configuration, but requires specialized equipment and skill and is time consuming.

Recently, approaches used for the determination of anomeric configuration of ribofuranosyl nucleosides have been critically reviewed.[4] In the following discussion, various commonly employed methods and criteria will be surveyed.

I. Baker's "trans rule"[5]

Baker's "trans rule", which states that "condensation of a heavy metal salt of a purine or pyrimidine with an acylated glycosyl halide will form a nucleoside with a

C-1' to C-2' trans configuration in the sugar moiety regardless of the original configuration at C-1'", has provided the initial basis for assignment of the anomeric nature of the products obtained from several coupling methods. The reason for the trans predominance has been attributed to the participation of the acyloxy group at C-2' resulting in formation of an orthoester ion involving C-1'. However, Montgomery and Hewson[6] have noted an exception to this rule. Fox and co-workers[7,8] observed that the "trans rule" could not be applied automatically to anomeric assignment of the products obtained from Hilbert-Johnson coupling reactions.

II. Chiroptical methods

1. Hudson's "isorotation rule"[9]

Hudson's "isorotation rule", which was the first attempt to utilize optical rotation data for determining the anomeric configuration of carbohydrates, was used along with Baker's "trans rule" for early assignments of the anomeric configuration of nucleosides. This empirical rule was originally based upon presumed relations between the anomeric configuration and optical rotations of carbohydrates. Although it remains applicable for most methyl glycosides,[10] it was found to fail for certain anomeric pyrimidine nucleosides.[11,12] Later the noted

exceptions to this rule were found to be general for most pyrimidine nucleosides.[13]

2. ORD and CD approaches

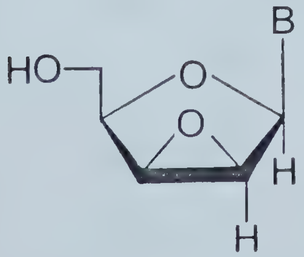
Ulbricht and co-workers[14] measured ORD and CD spectra of series of nucleosides and nucleotides and noted trends correlating the anomeric configuration and sign of the long-wavelength ("B_{2u}") Cotton effects. Purine β-D-nucleosides generally exhibit a negative Cotton effect and purine α-D-nucleosides a positive Cotton effect. Pyrimidine nucleosides generally exhibit reversed Cotton effects (β-D, positive, and α-D, negative). This so-called ORD/CD rule was in general harmony with experiments of Frič et al.[15] and Nishimura et al.[16] However, the sign of the Cotton effect as well as its magnitude is dependent[17,18] on intrinsic dipole vector orientations within the heterocyclic base, glycosyl rotameric populations, solvents, temperature, etc. Numerous exceptions to this rule have been found.[14a,c,f,18-20] Thus, it is noted[15,18b] that ORD and CD stereochemical assignments should be applied to nucleosides only in those cases where the unknown and reference nucleosides have similar transition moment directions, electronic structures in the sugar rings, and glycosyl rotameric populations.

III. NMR methods

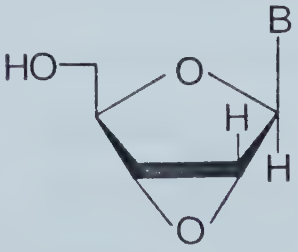
1. ^{1}H-NMR approaches

a. Coupling constant approaches

It is now generally recognized[21,22] that the coupling constant between H-1' and H-2' ($J_{1'-2'}$) should be $\lesssim$ 1.0 Hz for unequivocal assignment of a 1',2'-trans hydrogen relationship in the case of pentofuranosyl derivatives such as β-D-ribofuranosyl nucleosides (1). This criterion is based upon the Karplus equation,[23] by which the vicinal coupling constant is calculated from the dihedral angle.[24] However, $J_{1'-2'}$ > 1.0 Hz is common for 1',2'-trans hydrogen relationships because of the conformational flexibility of the furanose ring. No assignment is possible for nucleosides having the 1',2'-cis hydrogen relationship of α-D-ribofuranosylnucleosides (2) owing to the double-angle cosine dependence of the Karplus equation. The only exceptions to this trans $J_{1'-2'}$ < 1.0 Hz criterion have been observed[4] in the fused-ring 2',3'-anhydrolyxonucleosides (H-1' and H-2' cis) (3). The anomeric proton signal of (3) appears as a sharp singlet ($J_{1'-2'}$ < 0.7 Hz) as does the corresponding peak for H-1' of the 2',3'-anhydroribonucleosides (H-1' and H-2' trans) (4).



3

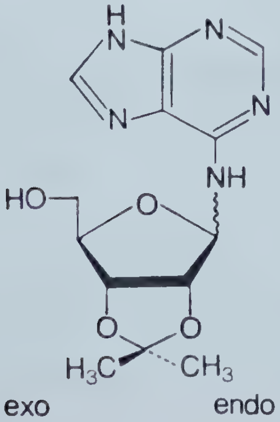


4

B = adenin-9-yl,
4-aminopyrrolo-
[2,3-d]pyrimidin-
7-yl

Leonard and Laursen[25] employed the 2',3'-O-isopropylidene group for ribonucleosides to restrict the furanose ring conformation and lower the coupling constant ($J_{1'-2'}$). Although the β-anomer usually responds to a reduction in $J_{1'-2'}$, values in the range of $J_{1'-2'} =$ 2~3 Hz are common, and unequivocal assignment is precluded.

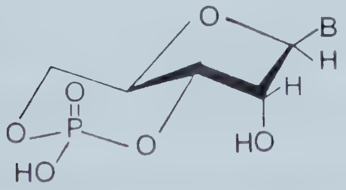
MacCoss et al.[26] proposed a "H-4' multiplicity criterion" (H-4' triplet for α-anomers and higher multiplicity for β-anomers). They investigated 12 anomeric pairs of 2',3'-O-isopropylidene ribonucleosides and attributed the H-4' multiplicity (coupling with H-3' as well as H-5', H-5") in the β-series to the flattened conformation of the 2',3'-O-isopropylidene ribose residue. The H-4' triplet (coupling with H-5' and H-5" only) in the α-series was related to the conformational change of the sugar residue resulting from interaction between the endo methyl group and the base reducing the dihedral angle between H-3' and H-4' ($\phi_{3'-4'}$) to ~90°. One observed exception was the anomeric pair of 6-N-(2',3'-O-isopropylidene-D-ribofuranosyl)adenines (5) which have increased flexibility around the glycosyl bond. Both α and β-anomers gave rise to triplet peaks for H-4'. Thus, a minimum



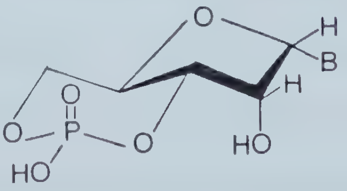
5

requirement of this rule, as they emphasized, is that the C-1' substituent should be of sufficient bulk and rigidity to ensure that the steric interaction between the 5'-CH_2OH group and the base of the β-anomers will prevent a decrease in $\phi_{3'-4'}$. The foregoing coupling approaches are crucially dependent on the sugar conformation. Conformational rigidity is a predominant factor in their applications.

Jardetzky[27] noted that $J_{1'-2'} < 1$ Hz for adenosine 3',5'-cyclic-monophosphate (cAMP) (6, B = adenin-9-yl). However the applicability of this observation to the anomeric assignment of ribonucleosides remained unrecognized until Robins and MacCoss[4,28] proposed a "geometry-only method" for this determination based upon the coupling of the anomeric proton ($J_{1'-2'}$) of the conformationally rigid anomeric 3',5'-cyclonucleotides (6,7). The proposed criterion was $J_{1'-2'} < 1$ Hz for β-anomers (6) and $J_{1'-2'} \geq 3$ Hz for α-anomers (7). They observed no exceptions in examining over 200 examples and suggested



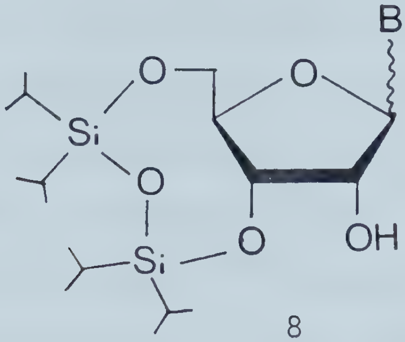
6



7

its general applicability to variously substituted ribo-3',5'-cNMP's (cyclic nucleoside monophosphates) and

β-ara-3',5'-cNMP's. Similar results have been observed[29] in our laboratory recently using the 3',5'-O-(1,1,3,3-tetraisopropylidisilox-1,3-diyl) group[30] (8) in place of the 3',5'-O-cyclic-monophosphate.



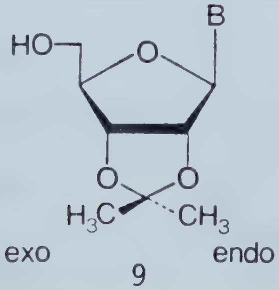
8

b. Chemical shift approaches

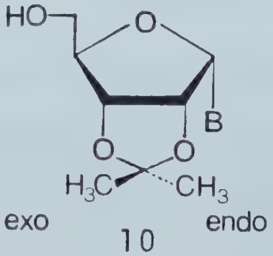
Fox and co-workers[31] reported a method based on the chemical shift difference of 2'-O-acetyl methyl signals before and after the hydrogenation of a double bond in the heterocyclic base. In pentofuranosyl nucleosides of pyrimidines, they observed a small diamagnetic (upfield) shift with 1',2'-trans and a large paramagnetic (downfield) shift with 1',2'-cis 2'-O-acetyl derivatives upon saturation of the 5,6-double bond. This approach is based upon the anisotropic effect of the heterocycle and appears to be generally applicable to pyrimidine nucleosides. However, it is not readily applicable to purine nucleosides since selective hydrogenation to give 7,8-dihydropurine nucleosides is difficult.

Montgomery[32a] reported that the 2'-*O*-acetyl methyl chemical shift *per se* could be used to determine the anomeric configuration. In a series of acetylated anomeric furanosyl purine pairs, it was observed that 2'-*O*-acetyl methyl signals of 1',2'-cis nucleosides occur upfield from δ 1.95 ppm while those of 1',2'-trans nucleosides occur downfield from δ 2.05 ppm. Similarly, 2'-*O* and 2'-*S*-methyl chemical shifts have been utilized for the anomeric configurational assignment of arabinofuranosyl nucleosides.[32b] However, the chemical shifts of acetyl methyl groups are solvent dependent[33] and an exception has been noted in the case of 1-(2,3,5-tri-*O*-acetyl-β-*D*-ribofuranosyl)benzotriazole.[34a]

Imbach and co-workers[34] proposed a very useful method for the configurational determination of ribofuranosyl nucleosides, which involves the chemical shift difference ($\Delta\delta$) of the two methyl signals (exo and endo) of anomeric 2',3'-*O*-isopropylidene ribofuranosyl nucleosides (9,10). The proposed $\Delta\delta$ criterion,[34b] as refined further,[34a,d] is: $\Delta\delta > 0.15$ ppm for the β-anomer (9) and $\Delta\delta < 0.15$ ppm for the α-anomer (10). This criterion is



9



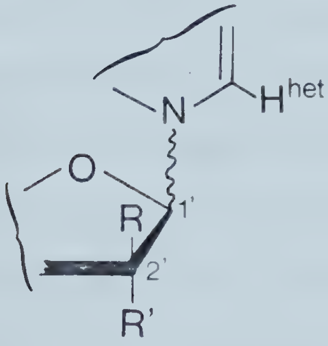
10

based upon the anisotropic effect of the heterocyclic "aglycon" on the chemical shift of the endo methyl group of the α-anomer. This anisotropy was demonstrated[4,34a-e] with an α-anomer of 2',3'-O-isopropylidene-5,6-dihydrouridine (10, B = 5,6-dihydrouracil-1-yl), which does not obey the rule. They have noted that the Δδ criterion should be limited to ribofuranosyl compounds having an unsaturated heterocyclic aglycon and no 5'-substituents. These limitations of the criterion are now well documented.[35-38] However, an exception to this restricted criterion has been reported for 1-(2,3-O-isopropylidene-D-ribofuranosyl)-3-carbamoyl pyridinium salts.[39]

Nishimura and Shimizu[40] noted that the anomeric proton of a 1',2'-trans (2'-OH to 1'-heterocycle) nucleoside appears at a higher field than that of the corresponding cis isomer. This chemical shift difference of the anomeric protons of α and β-nucleosides has been considered to result from the diamagnetic shielding[41] of the cis (H-1' to 2'-OH) 2'-OH group on the anomeric proton of 1',2'-trans nucleosides. This method has been used for anomeric configurational assignments and found consistent. However, it has been reported[37b] that the anomeric proton shifts of isopropylidene derivatives of anomeric mannofuranosylimidazole and mannofuranosyladenines are not in accordance with this rule. Thus,

caution must be exercised with 2'-OH protected nucleosides when this rule is applied. Furthermore, it is not applicable where only one anomer is available since this method requires both anomers for comparison.

Alenin and Domkin[42] recently proposed a new criterion for establishing the anomeric configuration of furanosyl derivatives of purines, pyrimidines, imidazoles and pyrazoles (11). They observed that when the heterocycle at C-1' is trans to the 2'-hydroxy group the chemical shift of the heterocycle proton (H^{het}) appears at lower field than that of H^{het} in a cis orientation. They combined the chemical shift of H^{het} with that of the anomeric proton for their proposed criterion which states that: the chemical shift difference between H^{het} and H-1 ($\Delta\delta = \delta H^{het} - \delta H\text{-}1'$) is always greater when the heterocycle and 2'-hydroxy group have a trans orientation. This relation appears to be valid for unprotected nucleosides and their isopropylidene derivatives but not for nucleosides



11

H^{het}; H^8 (purines), H^6 (pyrimidines)
H^2 (imidazoles), H^5 (pyrazoles)

R = H, R' = OH in ribo-, xylo- and glucofuranosyl nucleosides

R = OH, R' = H in arabinofuranosyl nucleosides

with acetyl, benzoyl and benzyl protecting groups on the sugar hydroxyls. A similar chemical shift difference of the heterocycle protons has been noted previously with the anomeric C-nucleosides of pseudocytidine.[43] This criterion is not confirmed with a large number of examples and it is not applicable to H^{het} substituted nucleosides. Again, both anomers are required.

c. Nuclear Overhauser effect (NOE) approach

Cushley et al.[44] reported the NOE method for determining the anomeric configuration of purine and pyrimidine nucleosides. They observed signal enhancements of the aglycon protons upon irradiation of H-4' of α-nucleosides, but no enhancement upon irradiation of H-5'. Irradiation of H-5' of β-nucleosides produced enhancements of the aglycon protons while irradiation of H-4' had no effect. However, NOE is dependent on rotameric populations and sugar ring conformations. Overlapping shifts of the sugar protons and coupling between these protons also complicate NOE measurements.

d. Relaxation time (T_1) approach

Hall and Preston[45] measured the relaxation times (T_1) of the anomeric protons of pyranosyl carbohydrates and observed a difference in the relaxation times (T_1) between α and β-anomers. The applicability of this

method to nucleosides has been demonstrated by Tran-Dinh et al.[46] They observed that the relaxation times (T_1) of the anomeric protons of the β-anomers of some anomeric C-nucleosides are about two and a half times longer than those of the α-anomers. However, this method requires more sophisticated techniques and calculations and is time consuming. It has not been reported with naturally occurring N-glycosyl nucleoside examples.

2. ^{13}C-NMR approaches

In 1974, Shimizu et al.[47] reported ^{13}C-NMR spectra of anomeric pyrimidine and purine ribonucleosides. They observed that the C-1' and C-2' chemical shifts of α-anomers appeared at higher fields (2~4 ppm) than those of the β-anomers. Similar chemical shift differences of the anomeric carbon (C-1') have been observed with anomeric 2',3'-O-isopropylidene ribofuranosyl C-nucleosides,[38] methyl glycosides[48] (anomeric ribo, arabino, xylo and lyxofuranosides), 2',3'-O-isopropylidene ribofuranosyl purine nucleosides[36] and arabinofuranosylcytosine.[32b] In all of the above cases, the anomeric carbon (C-1') of the nucleosides and glycosides having a cis relationship between the aglycon and 2'-substituent resonate at a higher field than that of the corresponding 1',2'-trans compounds.

The existence of correlations between chemical shifts

of the isopropylidene methyl groups of 2',3'-O-isopropylidene-D-ribofuranosyl derivatives and the anomeric configuration,[38,49] and of the central (or quaternary) isopropylidene carbon and the anomeric configuration[49a] have also been reported. At present, the reliability and generality of these observation in the determination of anomeric configuration has not been fully established and further investigation is needed.

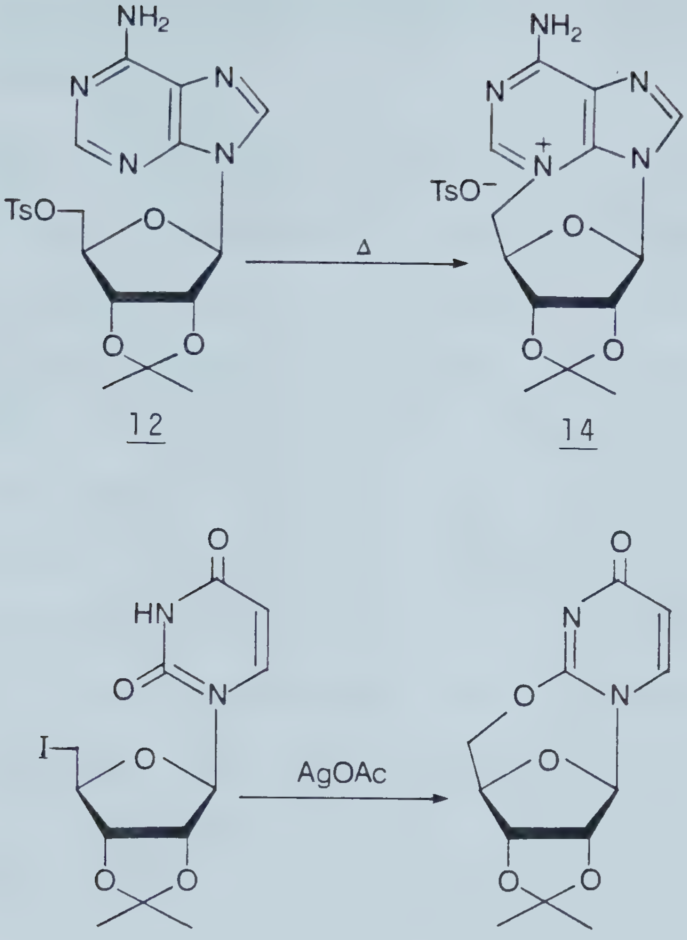
IV. Enzymatic methods

Enzymes such as bacterial nucleoside hydrolase,[50] pyrimidine nucleoside phosphorylase[8] and calf intestine deaminase[51] have been used to distinguish certain α and β-nucleosides. However, the general applicability is limited to the substrate specificity of the enzymes.

V. Chemical methods

1. Cyclonucleoside approach

Todd and co-workers[52] discovered a method to assign the glycosyl configuration unequivocally by the formation of cyclonucleosides linking the 5'-position of the sugar residue and the heterocyclic base, such as 14[52a] and 15.[52d] Such cyclization is possible only for 1',4'-cis (β-D or β-L) nucleosides (Scheme I). This formation of cyclonucleosides further established[52b,c] the anomeric

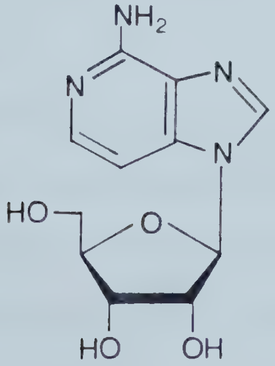


12 14

13 15

Scheme I

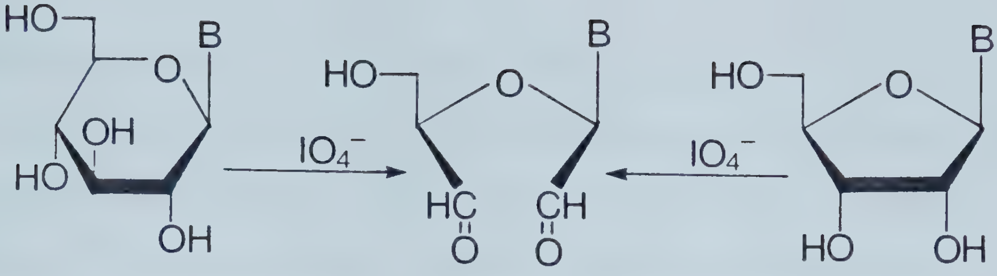
configuration of thymidine, 2'-deoxycytidine and 2'-deoxyadenosine as β. However, synthetic nucleosides without the requisite functionality for the formation of cyclonucleosides, such as 3-deazaadenosine (16),[53] are precluded from evaluation by this method.



16

2. Degradation approaches combined with optical rotations

Periodate (or periodic acid) oxidation, which Malaprade[54] was the first to introduce for selective oxidation of *vic*-glycols, is a widely used reaction[55] in the field of carbohydrate chemistry for structural studies.[56-59] This oxidation, in combination with enzymatic and chemical reactions, has also been used as a tool for sequence analysis of ribonucleic acid (RNA) and polynucleotides.[60-62] Early work on configurational assignments in the carbohydrate field was based on the optical rotations of the oxidation product (the dialdehyde).[63] Applications of this oxidation approach in nucleoside chemistry were used to establish the furanose structure of the sugar residue of naturally occurring ribonucleosides and later their anomeric nature.[64-67] The first indicative configurational assignment of nucleosides was carried out by Todd and co-workers[65] using periodate oxidation. They obtained the same dialdehydes (*18*) for a given pair of synthetic (*17*) and naturally occurring (*1*) nucleosides upon oxidation (Scheme II). Since the synthetic glucopyranosyl nucleosides (*17*) had been considered to have the β-configuration by their method of preparation, they assigned the anomeric configuration of *1* as β. Later the unambiguous confirmation

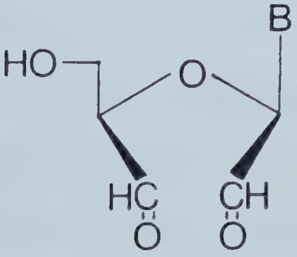


17 18 1

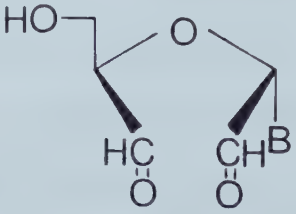
B = adenin-9-yl, uracil-1-yl cytosin-1-yl

Scheme II

of the β-configuration was demonstrated by the formation of cyclonucleosides[52] and by X-ray diffraction.[3] Similar use of optical rotational measurements on the periodate oxidation products (dialdehydes) derived from reference and unknown nucleosides has been made to establish the anomeric nature of other nucleosides[8,40,67-73] and C-glycosides[74] and to determine their anomeric purity.[75] However, there remains some uncertainty in the anomeric configurational assignment based on the optical rotations of these aldehydes. These compounds, such as 18 and 19, contain two asymmetric centers and can exist as diastereomers. Identical rotations have been reported[76] for

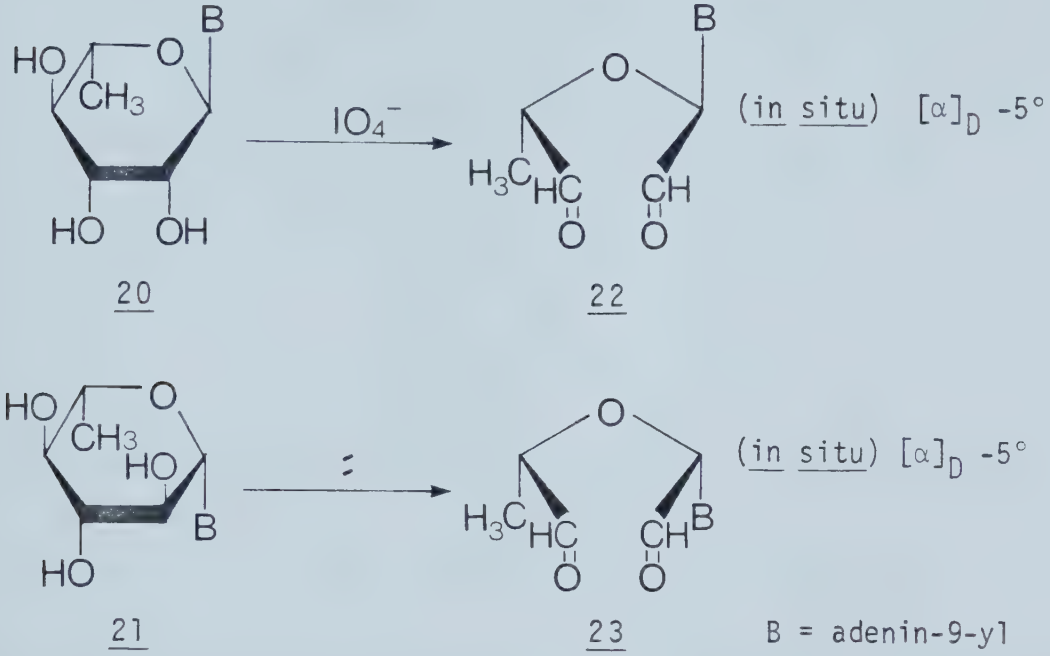


18



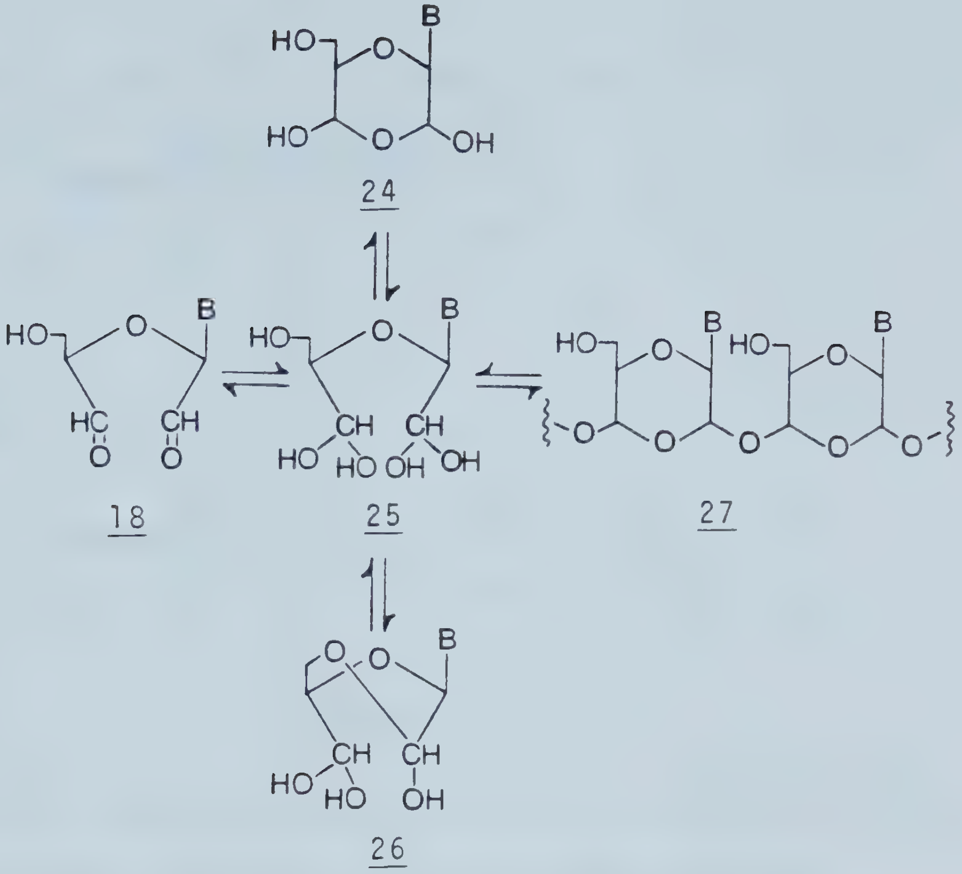
19

the dialdehydes (22,23) obtained *in situ* by periodate oxidation of 9-(6-deoxy-α-L-mannopyranosyl)adenine (20) and 9-(6-deoxy-β-L-glucopyranosyl)adenine (21), even though the dialdehydes 22 and 23 are diastereomers (Scheme III).



Scheme III

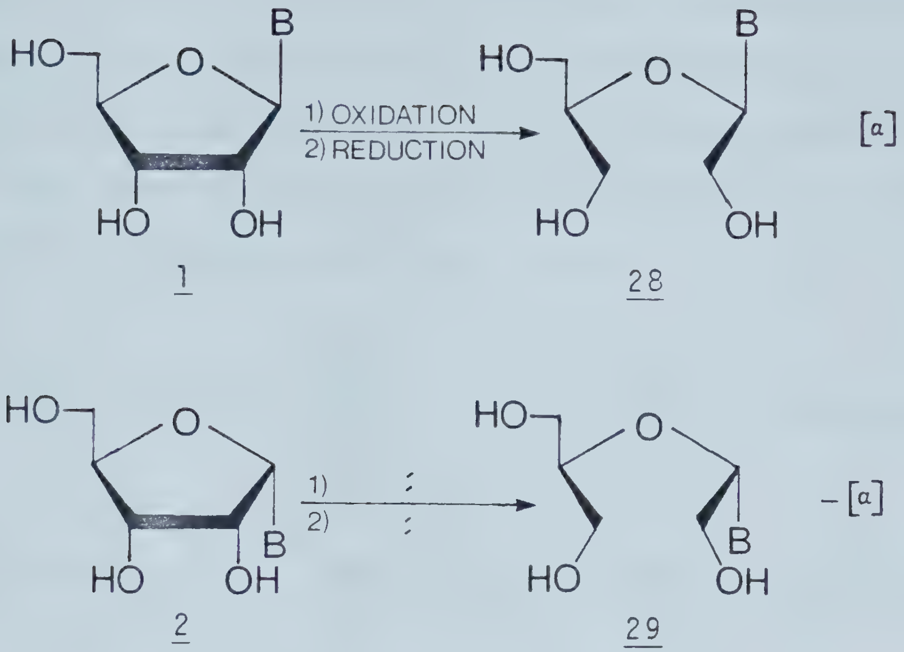
The dialdehydes from common naturally occurring ribonucleosides have been isolated and characterized[65,66,77-79] usually as derivatives. It is well established[59,78,79] that dialdehyde such as 18 exists in solution as hydrated species, internal hemiacetals, and hemialdals (24-26). Only a small fraction of these compounds exist as the free aldehydes (Scheme IV). In the solid state, the "dialdehydes" were suggested[78c] to



Scheme IV

be completely polymerized (27). The internal hemiacetal (26) and hemialdal (24) forms create additional asymmetric centers. Therefore, cases in which the optical rotations of periodate oxidation products provide sufficient information to allow unequivocal assignment of the anomeric configuration are rare.

Reduction[77] of these oxidation products with hydrogen and Raney nickel or borohydride normally gives the corresponding tri-alcohols such as 28 and 29 (Scheme V). Partial reduction of the aldehyde function distal to the aglycon has been noted under controlled conditions.[77,80b] This

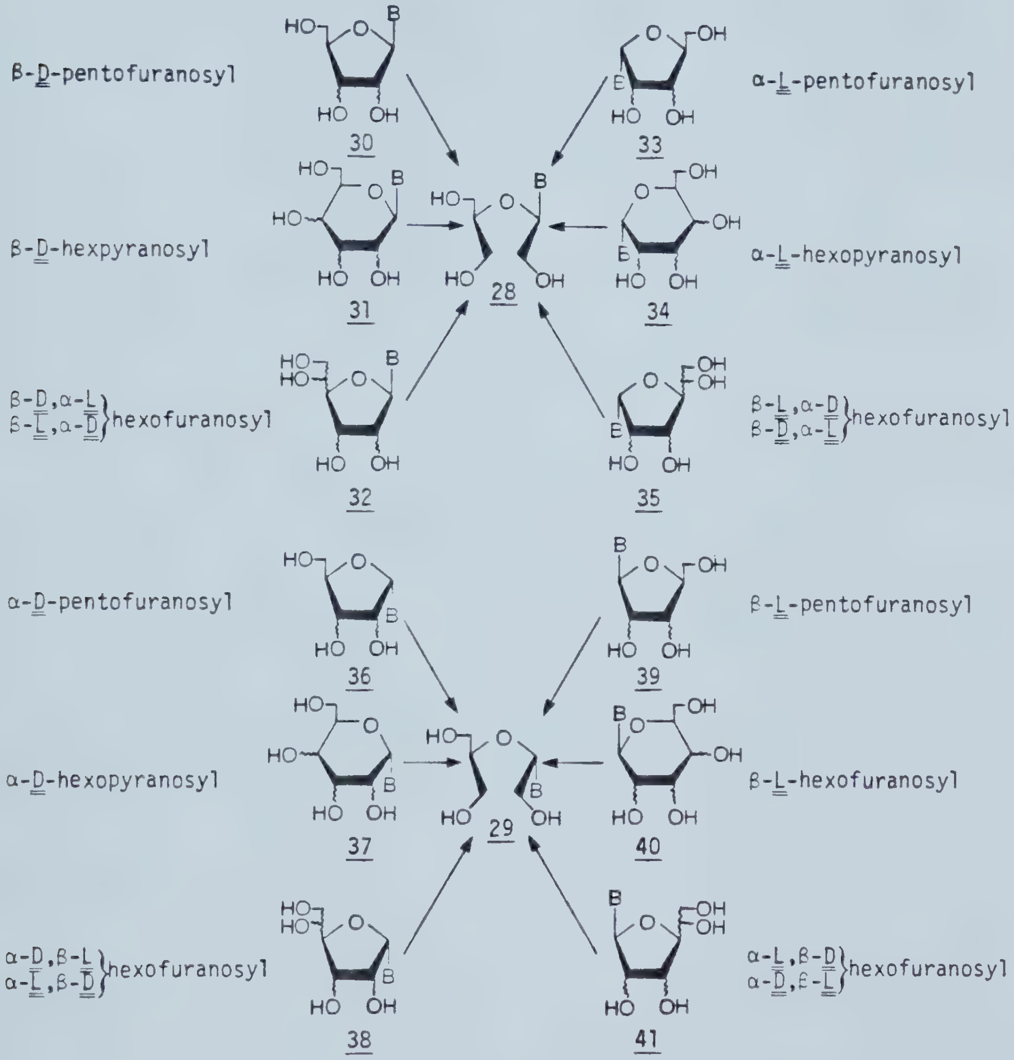


Scheme V

is a superior method for correlating the anomeric configuration of the precursor nucleoside since the only asymmetric center remaining in the reduced compound (28 or 29) is the original anomeric carbon. The triols (28, 29) are now enantiomeric and give equal but opposite rotations so that the anomeric natures of the original nucleosides (1,2) are clearly revealed.

Smith et al.,[80] who extended the work of Hudson,[63] and other workers[81] demonstrated the configurational correlation of methyl glycosides in both the D and L-series based on the rotations of oxidized and reduced products (28,29, B = OMe). This method for determining the anomeric configuration requires a reference compound of known anomeric configuration. However, a principal

advantage is its applicability to a large number of compounds (some of which are shown in Scheme VI). Not only the anomeric configuration but also D and L-series can be correlated and determined[80,81] from rotation measurements on precursor and triol series.



Scheme VI

In 1958 Khorana and co-workers[50] introduced the foregoing method to nucleosides in their synthesis of α-adeno-

sine. Since then, a number of examples has appeared. A trend in the optical rotations of oxidized and reduced nucleosides (nucleosides$_{\text{ox-red}}$) has been observed relative to the anomeric configuration of the starting nucleosides (see next section). In the usual procedure a nucleoside$_{\text{ox-red}}$ is prepared *in situ* by the periodate oxidation and subsequent reduction of the nucleoside in question. The optical rotation of the resulting quantitated solution is then measured. When this method is used, caution must be exercised to avoid over-oxidation, and nucleosides that are labile to the degradation conditions also have been reported.[82-89]

The various methods described above should be evaluated carefully and exceptions and limitations considered before they are employed to determine the anomeric configuration of nucleosides. In this thesis, the latter degradation method (oxidation and reduction) for nucleosides, which had not been studied systematically, will be investigated.

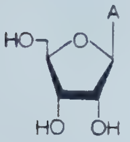
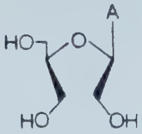
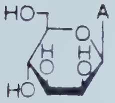
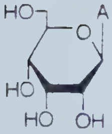
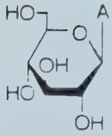
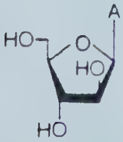
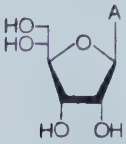
RESULTS AND DISCUSSION

Since Khorana et al.[50] first employed the furanose degradative procedure (oxidation of the glycol with periodate and reduction of the derived dialdehyde with borohydride) for configurational assignment of α-adenosine, a number of examples has appeared. This method has been used for confirming the anomeric relation of nucleoside pairs as well as for direct anomeric configurational assignment. Examples are summarized in Table I for in situ prepared nucleosides$_{\text{ox-red}}$ and isolated "fraudulent" (sugar-modified) nucleosides$_{\text{ox-red}}$. Table II contains data for the isolated nucleosides$_{\text{ox-red}}$ of "natural" triol types (28 or 29).

Lerner had observed[96,97] that nucleosides having a β-D or α-L configuration gave ox-red alcohols with positive optical rotations, and nucleosides having an α-D or β-L configuration gave ox-red alcohols with negative optical rotations. On this basis, assignment of the anomeric configuration of adenine nucleosides such as 70 and 82 in Table I (which presumably give the corresponding nucleosides$_{\text{ox-red}}$ 71 and 83, respectively, with more than one asymmetric center) has been proposed.[96,97] It can be seen from Table I and Table II that Lerner's empirical approach agrees with the examples reported with one exception. The enantiomeric diols (96 and 98) gave opposite relations to those expected. However, optical rotations of the

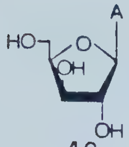
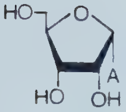
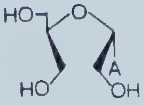
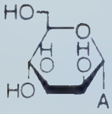
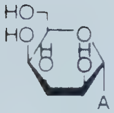
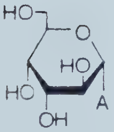
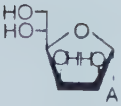
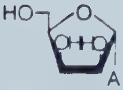
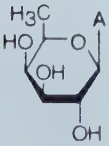
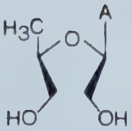
TABLE I

Optical Rotations of Previously Reported *in situ* Prepared and Isolated Nucleosides$_{ox-red}$

Precursor nucleosides	Oxidized-reduced products (nucleosides$_{ox-red}$) b)	$[\alpha]_D$ c)	References
 a) 42	 43	+61-68	37,50,90,91
 44	43	+57	92
 45	43	+58	92
 46	43	+57	92
 47	43	+66	90
 48	43	+70	37

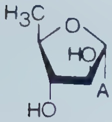
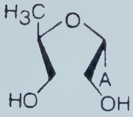
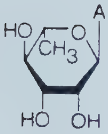
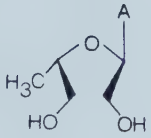
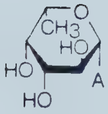
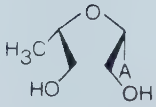
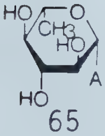
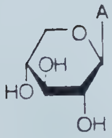
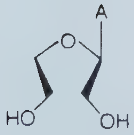
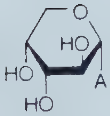
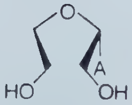
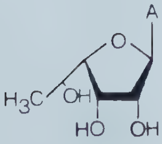
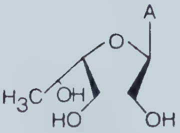
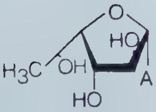
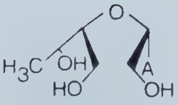
continued.....

TABLE I (continued)

 49	43	+53	93
 50	 51	-66	50
 52	51	-58	92
 53	51	-59	92
 54	51	-57	92
 55	51	-64	37
 56	51	-65.0	91
 57	 58	+55 (isolated)	94

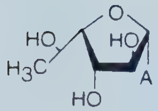
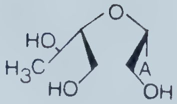
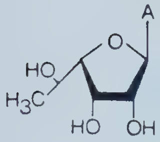
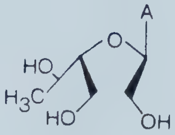
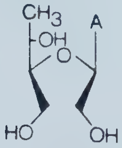
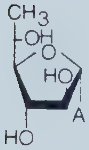
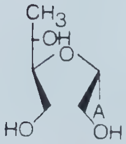
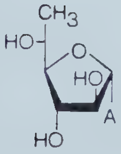
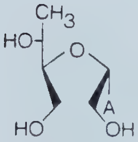
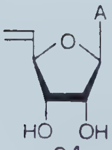
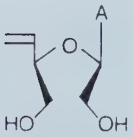
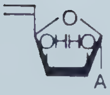
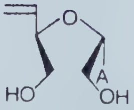
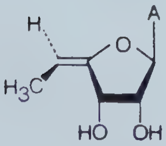
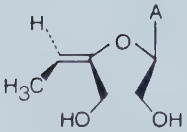
continued.....

TABLE I (continued)

 59	 60	-71	95
 61	 62	+74 +80.6 (isolated)	76,95 94
 63	 64	-46 -54 (isolated)	76 94
 65	64	-50	76
 66	 67	+34.5 (isolated)	94
 68	 69	-36.5 (isolated)	94
 70	 71	+37	96
 72	 73	-64	96

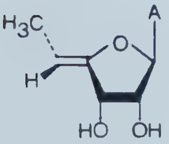
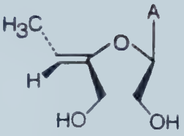
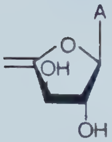
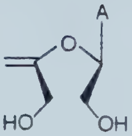
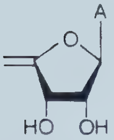
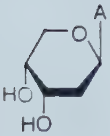
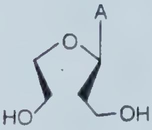
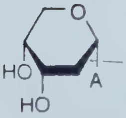
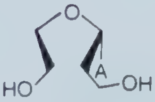
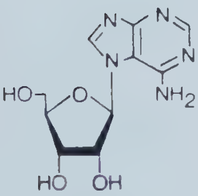
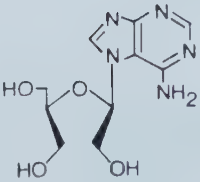
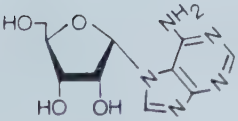
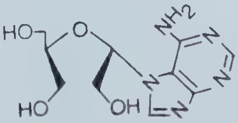
continued.....

TABLE I (continued)

 74	 75	-69	96
 76	 77	+65	96
 78	 79	+72	97
 80	 81	-48	97
 82	 83	-62	97
 84	 85	+37	98
 86	 87	-60	98
 88	 89	+18	35

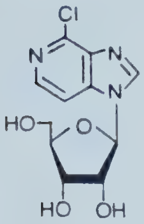
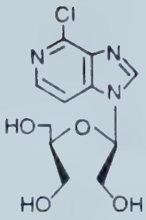
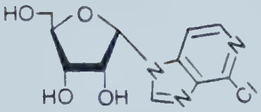
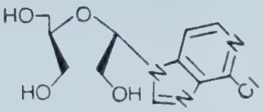
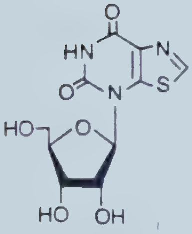
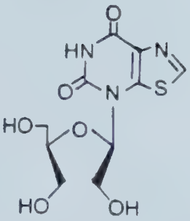
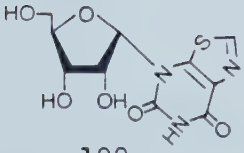
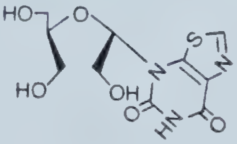
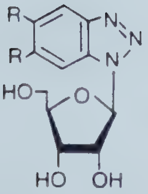
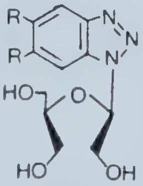
continued.....

TABLE I (continued)

 90	 91	+18	35
 92	 93	+59	99
 94	93	+55	99
 95	 96	-10.8	100
 97	 98	+10.2	100
 99	 100	+118.1±0.2	101
 101	 102	-117.9±0.2	101

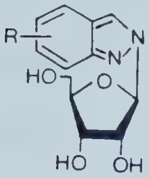
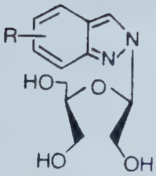
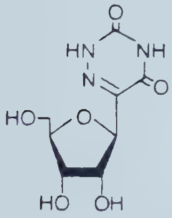
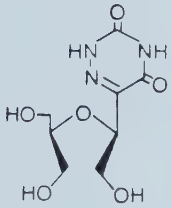
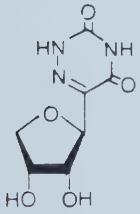
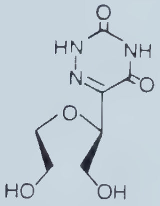
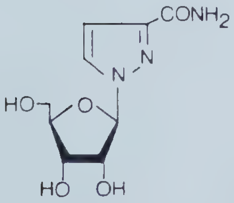
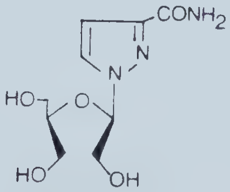
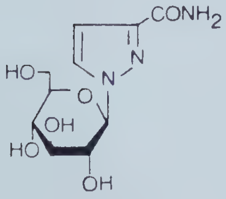
continued.....

TABLE I (continued)

 103	 104	+72.0	53
 105	 106	-71.9	53
 107	 108	+73.5	102
 109	 110	-75.0	102
			103
111 R=CH_3	114 R=CH_3	+65.1	
112 R=Cl	115 R=Cl	+67.2	
113 R=H	116 R=H	+60.7	

continued.....

TABLE I (continued)

			104
117 R=6-NO_2	121 R=6-NO_2	+14.9	
118 R=5-NO_2	122 R=5-NO_2	+ 7.6	
119 R=H	123 R=H	+15.2	
120 R=3-CN	124 R=3-CN	+51.3	
 125	 126	+46.8	105
 127	 128	+39.2	106
 129	 130	$+36.1^{d)}$	107
 131	130	$+31.8^{d)}$	107

---

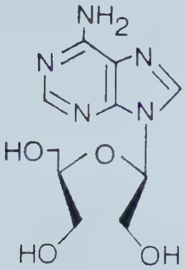
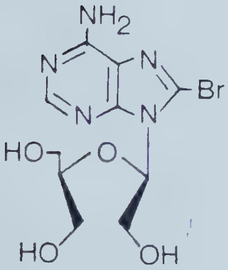
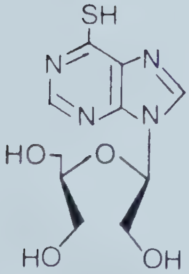
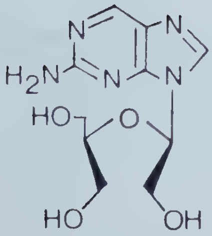
continued.....

Table 1 (continued)

a) A = adenin-9-yl. b) Structures, if not reported were presumed. c) Rotation values are of *in situ* prepared nucleosides$_{ox\text{-}red}$ unless noted otherwise. Consult the original article for detailed measurement conditions (i.e. temperature etc.). d) The reported $[M]_D$'s were converted to $[\alpha]_D$'s.

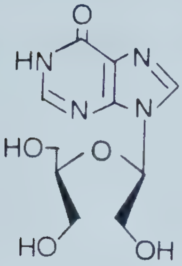
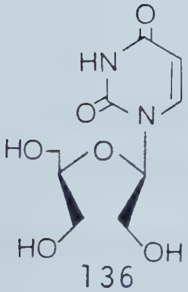
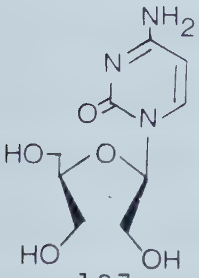
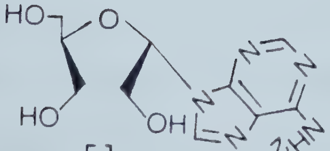
TABLE II

Optical Rotations of Previously Reported Isolated Triols (28, 29)

Compounds	$[\alpha]_D$ a)	Reference
 43	+58.9	108
 132	+38	109
 133	+73	109
 134	+34	109

continued.....

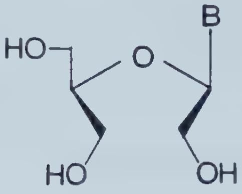
Table II (continued)

 135	+70.2	109
 136	+49±1	108
 137	+62±1	108
 51	-62.6	94

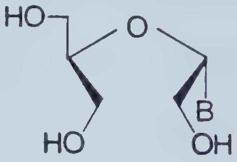
---

a) Specific rotation values in H_2O. Consult the original article for detailed conditions (i.e. temperature etc.).

"natural" triols (28 and 29), which can be derived from anomeric pentofuranosyl, hexopyranosyl and hexofuranosyl nucleosides, are in accord with expectations.



28

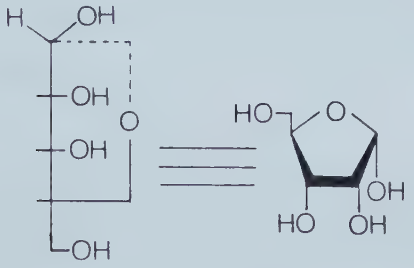


29

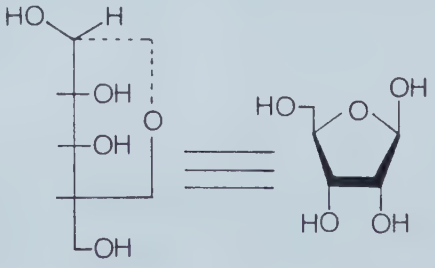
Despite the well-known use of such triols,[50] this empirical approach has not been investigated systematically. Therefore, we have studied optical rotation relationships of various types of derived triols prepared *in situ* from pentofuranosyl nucleosides. We have observed that they exhibit a predictable electrophoresis behavior under defined complexing conditions. We have measured optical rotation values for a series of adenosine$_{ox-red}$ derivatives to evaluate substituent effects. The variation in optical rotations of some isolated nucleosides$_{ox-red}$ with pH, and some significant solvent effects have been determined. The rates of periodate reduction with some anomeric pairs of D-ribofuranosyl nucleosides, and with some pentofuranosyl adenine and cytosine nucleosides having a sugar configuration other than ribofuranose have been evaluated qualitatively.

A NOTE ON THE DEFINITION OF α AND β CONFIGURATIONS

In 1909 Hudson[9] suggested that the more dextrorotatory anomer in the D-series be designated α and the more levorotatory β. In the L-series the more levorotatory would be designated α and the more dextrorotatory β. Freudenberg[111a] suggested in 1932 that α and β anomers be classified with regard to their configuration rather than the sign and value of their optical rotations. His designation correlated monosaccharides with the same configuration at the anomeric carbon atom (C-1 for aldose) and the highest numbered asymmetric carbon (C-4 for pentoses) as α. Thus, structures (138 and 139), depicted in the Fischer and Haworth projection formulas, represent α-D-ribofuranose and β-D-ribofuranose, respectively. Their mirror images are α-L and β-L-ribofuranose.



138



139

In the majority of cases, both the Hudson and Freudenberg conventions concur. However, examples[11,12] are known of α-D compounds that are less dextrorotatory than their

anomers. Hence, Freudenberg conventions are now universally applied to glycosidic and glycosyl compounds. The pairs of such compounds which differ in configuration at the hemiacetal carbon atom (C-1 for aldoses) were termed "anomers" by Riiber.[111b]

A NOTE ON THE NOMENCLATURE OF OXIDIZED-REDUCED NUCLEOSIDES (NUCLEOSIDES$_{\text{OX-RED}}$) OF TRIOL TYPES (28 AND 29)

There are several ways of naming nucleosides$_{\text{ox-red}}$ (28 and 29).[108-110] For instance oxidized-reduced adenosine (43) has been named as 1-*O*-(1,3-dihydroxy-2-propyl)-1-(adenin-9-yl)-1(*R*),2-ethanediol, as 2-*O*-[1(*R*)-(9-adeninyl)-2-hydroxyethyl]glycerol and as 2-(*R*)-(adenin-9-yl)-4-hydroxymethyl-3-oxapentan-1,5-diol. For the sake of clarity in this thesis, oxidized-reduced nucleoside will be informally termed as nucleoside$_{\text{ox-red}}$ such as adenosine$_{\text{ox-red}}$ for 43. When prepared *in situ*, nucleoside$_{\text{ox-red}}$ (*in situ*) will be used.

OPTICAL ROTATIONS OF NUCLEOSIDES$_{\text{OX-RED}}$ (*in situ*); AND ELECTROPHORESIS OF NUCLEOSIDES$_{\text{OX-RED}}$ (*in situ*) AND THEIR PRECURSOR NUCLEOSIDES

6-Azauridine (182), 6-azacytidine (183), 3-methyl-6-azauridine (184), 3-methyl-6-azauridine$_{\text{ox-red}}$ (185), 6-methyl-2-β-D-ribofuranosyl-3-pyridazone (188), 2-β-D-ribofuranosyl-3-pyridazone (189), 6-methyluridine (173),

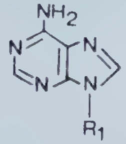
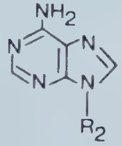
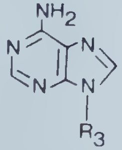
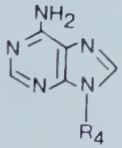
6-methylcytidine (178), 3-β-D-ribofuranosyl-6-methyluracil (171), 1,3-bis(β-D-ribofuranosyl)-6-methyluracil (172), 1,3-dimethylpseudouridine (190), 1-methylformycin (155), 2-methylformycin (156), 2-methylformycin$_{\text{ox-red}}$ (157), 5,6-dihydrouridine (187), α-L-uridine (163), β-L-uridine (164) and α-L-cytidine (180) were prepared by reported methods or by the general synthetic procedures developed for the present study (see Experimental Section).

Fifty four pentofuranosyl nucleosides and methyl β-D-ribofuranoside were oxidized with periodate and subsequently reduced with sodium borohydride. Excess sodium borohydride was destroyed with 1 *M* sodium dihydrogen phosphate buffer (pH 6.5). (Liberation of iodine was observed in several cases when 10% acetic acid was used to destroy the borohydride.) The reduction products were assumed to be the triols 28 or 29, depending upon the precursor nucleosides (see Scheme VI). This appears to be justified based on the quantitative nature of the reactions and the comparison of electrophoretic mobilities of nucleosides$_{\text{ox-red}}$ (*in situ*) with those of the corresponding precursor nucleosides.

Optical rotations of all nucleosides$_{\text{ox-red}}$ (*in situ*) and some isolated nucleosides$_{\text{ox-red}}$ were recorded at the wavelength of the sodium D line (589 nm). ORD curves (300~600 nm) and/or optical rotations at wavelengths other than 589 nm (578, 546, 436 and 365 nm bands of mercury)

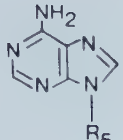
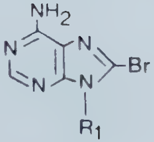
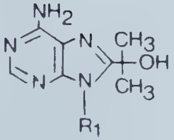
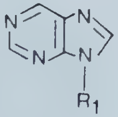
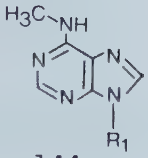
TABLE III

Optical Rotations of Nucleosides$_{\text{ox-red}}$ (*in situ*), and Electrophoresis of Nucleosides$_{\text{ox-red}}$ (*in situ*) and Their Precursor Nucleosides Determined in the Present Study

Precursor Nucleosides	$[\alpha]_{365}$	$[\alpha]_{436}$	$[\alpha]_{546}$	$[\alpha]_{578}$	$[\alpha]_{589}$	Anodic a) migration of nucleosides$_{\text{ox-red}}$ (*in situ*)	Anodic a) migration of precursor nucleosides
 42	plain positive curve b)				+65.6	0.38	0.62
 50	-203.8	-130.6	-77.0	-67.9	-65.2	0.38	0.62
 140	+199.0	+128.2	+75.7	+67.2	+63.9	0.38	0.67
 47	+177.0	+114.2	+67.4	+59.4	+56.8 c)	0.38	0.12

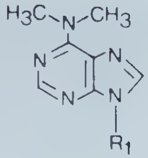
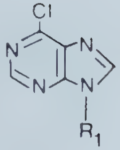
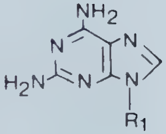
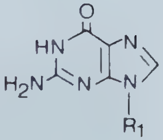
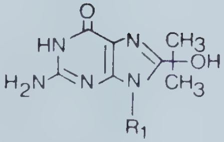
continued.....

Table III (continued)

 49	+182.4	+116.6	+68.3	+59.7	+57.7[c)]	0.38	0.60
 141	positive Cotton effect curve [b)]				+33.9	0.36	0.59
 142	———— [d)]				+70.0	0.40	0.60
 143	———— [d)]				+57.8	0.39	0.78
 144	plain positive curve [b)]				+60.4	0.52	0.81

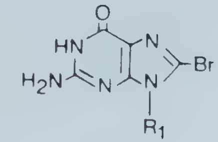
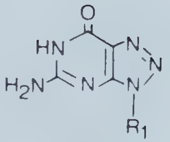
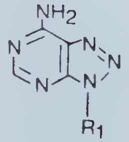
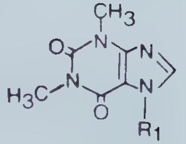
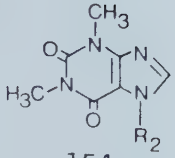
continued.....

Table III (continued)

 145	plain positive curve b)				+62.9	0.49	0.78
 146	+190.3	+121.3	+73.3	+65.0	+61.5	0.57	0.88
 147	+99.3	+75.8	+48.0	+43.0	+41.4	0.29	0.48
 148	positive Cotton curve b)				+40.4	0.64	0.83
 149	+468.9	+278.9	+155.9	+137.5	+131.3	0.53	0.74

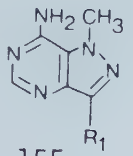
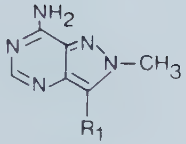
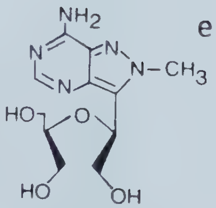
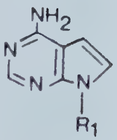
continued.....

Table III (continued)

 150	plain positive curve [b)]				+61.6	0.80	0.90
 151	+3.8	+14.6	+13.4	+11.5	+9.6	0.82	1.10
 152	+87.2	+55.7	+30.4	+26.7	+21.0	0.45	0.74
 153	+273.5	+154.4	+86.8	+76.5	+73.5	0.46	0.83
 154	-269.4	-152.1	-87.6	-76.7	-72.7	0.47	0.84

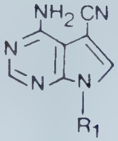
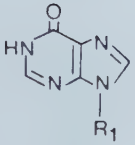
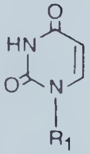
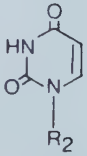
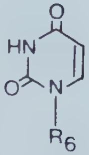
continued.....

Table III (continued)

 155	+173.5	+111.0	+64.1	+56.4	+54.0	0.38	0.64
 156	+115.5	+98.0	+62.0	+55.2	+53.0	0.45	0.67
 e) 157	+130.0	+107.4	+67.6	+59.8	+56.8	0.45	—
 158	positive Cotton effect curve, b) cross over at 302 nm				+32.3	0.41	0.52

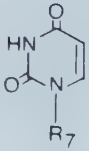
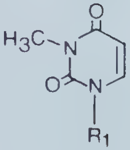
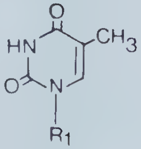
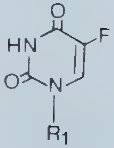
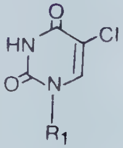
continued.....

Table III (continued)

 159	positive Cotton effect curve b)				+61.0	0.31	0.57
 160	plain positive curve b)				+70.3	0.81	1.07
 161	positive Cotton effect curve b)				+50.5	0.71	1.00
 162	negative Cotton effect curve b)				-51.4	0.71	1.00
 163	+114.2	+87.0	+56.8	+50.3	+49.1	0.71	1.00

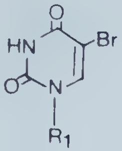
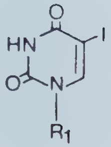
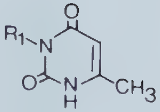
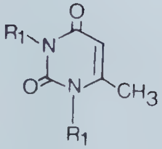
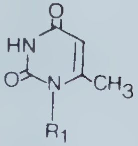
continued.....

Table III (continued)

 164	-117.6	-89.0	-58.2	-52.8	-46.2	0.71	1.00
 165	+132.4	+94.0	+59.1	+52.2	+49.7	0.55	0.92
 166	positive Cotton effect curve [b)]				+55.8	0.50	0.87
 167	+169.5	+107.7	+63.6	+55.2	+51.3	0.98	1.23
 168	+239.5	+158.2	+96.4	+84.8	+80.4	0.91	1.15

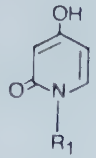
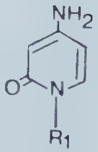
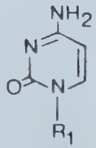
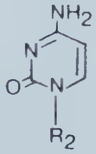
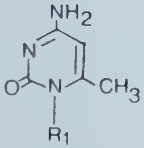
continued.....

Table III (continued)

 169	+196.5	+126.4	+76.9	+68.1	+64.3	0.95	1.10
 170	+250.6	+158.2	+90.6	+80.6	+74.8	0.86	1.15
 171	+16.3	+23.8	+18.5	+16.8	+16.3	0.49	0.90
 172	+102.5	+70.6	+43.5	+39.0	+35.9	0.63	1.15
 173	+205.9	+125.3	+72.1	+63.3	+60.8	0.63	0.93

continued.....

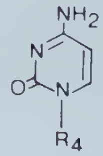
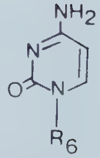
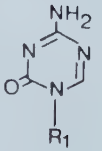
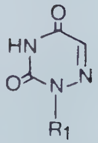
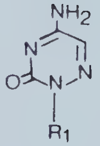
Table III (continued)

 174	positive Cotton effect curve, cross over at 306 nm [b)]				+53.7	0.89	1.12
 175	positive Cotton effect curve, cross over at 306 nm [b)]				+56.7	0.56	0.79
 176	positive Cotton effect curve [b)]				+62.2	0.54	0.88
 177	negative Cotton effect curve [b)]				-61.7	0.54	0.88
 178	+174.2	+107.2	+62.7	+55.4	+53.0	0.50	0.83

continued.....

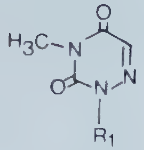
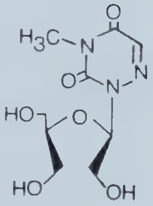
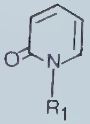
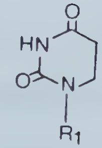
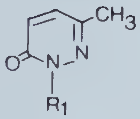
Table III (continued)

f)

 179	+157.0	+113.1	+71.1	+63.6	+60.4	0.53	0.17
 180	+151.1	+109.3	+68.3	+61.0	+58.0	0.53	0.87
 181	+74.5	+42.1	+25.8	+22.3	+19.9	0.45	0.82
 182	-148.7	-69.9	-30.7	-25.9	-25.7	1.00	1.40
 183	-226.7	-92.3	-38.5	-31.9	-30.1	0.41	0.82

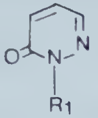
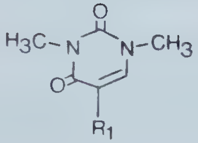
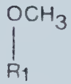
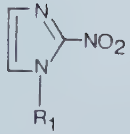
continued.....

Table III (continued)

 184	-131.9	-58.8	-26.1	-22.0	-20.6	0.48	0.93
 e) 185	-162.3	-73.4	-33.6	-27.8	-26.4	0.48	——
 186	positive Cotton effect curve, b) cross over at 355 nm				+52.9	0.51	0.90
 187	+167.9	+109.4	+65.4	+58.2	+55.1	0.45	0.88
 188	-419.3	-145.3	-53.8	-43.6	-40.6	0.43	0.90

continued.....

Table III (continued)

 189	-568.3	-225.3	-95.3	-80.9	-74.3	0.51	0.88
 190	+317.7	+194.3	+111.9	+98.4	+93.8	0.63	0.94
 191	plain positive curve b)				+12.5	——	——
 192	—— g)	+39.1	+105.1	+96.6	+93.4	0.46	0.84

R_1 = β-D-ribofuranosyl; R_2 = α-D-ribofuranosyl; R_3 = β-D-lyxofuranosyl; R_4 = β-D-arabinofuranosyl; R_5 = β-D-xylofuranosyl; R_6 = α-L-ribofuranosyl; R_7 = β-L-ribofuranosyl.

continued.....

Table III (continued)

a) Migrations were measured relative to that of uridine (1.00) as reference (absolute anodic migration of uridine ~12 cm). Values are from single determinations. b) Measured between wavelengths of 300 to 600 nm. c) These oxidations were allowed to proceed for 4 days. The lower rotation values observed possibly resulted from partial decomposition. d) Not measured owing to scarcity of samples. e) Isolated nucleoside$_{\text{ox-red}}$. f) HCl salt was used. g) No rotation value was obtained due to the strong absorption of light at this wavelength.

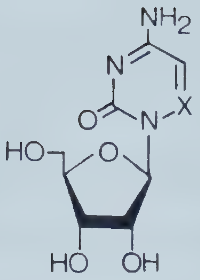
were also obtained for isolated nucleosides$_{\text{ox-red}}$ and most nucleosides$_{\text{ox-red}}$ (*in situ*). Electrophoretic mobilities of nucleosides$_{\text{ox-red}}$ and precursor nucleosides were recorded. The results are summarized in Table III. All measured nucleosides$_{\text{ox-red}}$ (*in situ*) and isolated$_{\text{ox-red}}$ gave plain positive or negative curves, or single positive or negative Cotton effects between 365 and 589 nm.

Lerner's results would predict that the triols **28** should give positive and triols **29** negative rotations at 589 nm. Our results for most of the nucleosides$_{\text{ox-red}}$ follow this trend. Thus, the anomeric pairs of nucleosides (**42** and **50**, **153** and **154**, **161** and **162**, **163** and **164**, **176** and **177**) in the D and/or L-series give positive rotations for β-D and α-L-nucleosides$_{\text{ox-red}}$ (**28**) and negative rotations for α-D and β-L-nucleosides$_{\text{ox-red}}$ (**29**). The results of nucleosides$_{\text{ox-red}}$ (*in situ*) derived from **141**-**150**, **165**-**170**, **173**, **178** and **187**, indicate that substituent effects and a minor change of the aglycon do not alter the sign of the rotation although the magnitudes are affected.

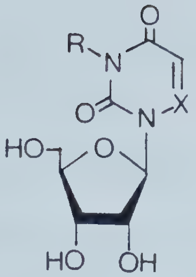
Purine nucleosides and their analogs appear to follow the trend. This is also the case for most of the pyrimidine nucleosides and their analogs and also for azomycin β-D-riboside (**192**) and methyl β-D-ribofuranoside (**191**). However, exceptions were found with the 6-azapyrimidine (1,2,4-triazene) and pyridazine nucleosides$_{\text{ox-red}}$. The ox-red products from 6-azauridine (**182**), 6-azacytidine

(183), 3-methyl-6-azauridine (184), 6-methyl-2-β-D-ribofuranosyl-3-pyridazone (188) and 2-β-D-ribofuranosyl-3-pyridazone (189) gave negative $[\alpha]_D$ values and plain negative curves between 365 and 589 nm, although these compounds were derived from β-D-ribofuranosyl nucleosides. This reversed trend was also reported with simple ox-red N-acetylglycosylamines[112] (28 and 29, B = N-acetylamino), whereas the methyl glycosides[80,81] (28 and 29, B = OMe) behaved normally.

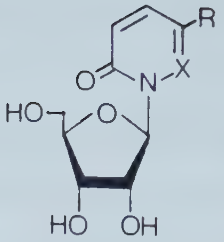
Initially a commercial sample of 6-azauridine was used for our study. However, the observation of a reversed sign for 6-azauridine$_{\text{ox-red}}$ led us to synthesize several 6-azapyrimidine and pyridazine nucleosides (182-184, 188 and 189). These compounds were reported to have CD and ORD spectra with reversed Cotton effects relative to their pyrimidine and pyridine counterparts.[14a-c,f,18e,113] The same structural relationship exists between pyrimidine and 6-azapyrimidine nucleosides as between pyridine and pyridazine nucleosides. Likewise, the rotations observed for the ox-red pyrimidine and pyridine nucleosides (161, 176, 165 and 186) were positive whereas those from their aza analogs (182, 183, 184 and 189, respectively) were negative. Isolated 3-methyl-6-azauridine$_{\text{ox-red}}$ (185) showed a parallel negative rotational pattern with the *in situ* result. This corroborated the structure of 3-methyl-6-azauridine$_{\text{ox-red}}$ (*in situ*) as the triol and



176; X = CH
183; X = N



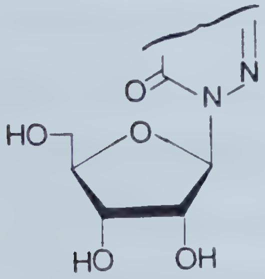
161; X = CH, R = H
165; X = CH, R = CH_3
182; X = N, R = H
184; X = N, R = CH_3



186; X = CH, R = H
188; X = N, R = CH_3
189; X = N, R = H

confirmed its negative rotation. Thus, the empirical trend clearly is not followed by 6-azapyrimidine and pyridazine nucleosides.

The common structural feature of the 6-azapyrimidine and pyridazine nucleosides (182-184, 188 and 189) consists of a six membered ring aglycon in which a second ring nitrogen atom and a carbonyl group are bonded to the ring nitrogen bearing the glycosyl moiety (193). The reversed sign of the Cotton effect of 6-azapyrimidine nucleosides was rationalized[18e] by suggesting a preferred conformation of the base. This was presumed to result from the interaction of the lone electron pair on N-6 and the lone pair on the ether oxygen of the ribose ring. If this were valid for the open-chain nucleosides$_{\text{ox-red}}$, it might also explain the abnormal



193

behavior of 6-azapyrimidine and pyridazine nucleosides$_{\text{ox-red}}$.[114]

However, 5-β-D-ribofuranosyl-6-azauridine (125, Table I), which has an adjacent carbonyl function and a nitrogen atom at the same ring position relative to the atom bearing the ribosyl moiety as 6-azapyrimidine nucleosides, has been reported to give the ox-red product (presumably 126) with a positive rotation.[105] Furthermore, a much greater conformational flexibility around the glycosyl bond of a nucleoside$_{\text{ox-red}}$ would be expected. This is supported by the "normal" positive optical rotations of nucleosides$_{\text{ox-red}}$ (*in situ*) derived from 6-methyluridine (173), 6-methylcytidine (178), 3-β-D-ribofuranosyl-6-methyluracil (171), and 8-(α-hydroxyisopropyl)guanosine (149). In contrast, the latter intact nucleosides represent exceptions from the ORD/CD rule (see the Introduction) and give a reversed Cotton effect relative to their unsubstituted pyrimidine and purine analogs.[14c,18b,f,e] These reversed Cotton effects have been attributed to preferred base conformations (restricted ranges of the torsion angles, ϕ_{CN}[115]) resulting from coulombic repulsion between base carbonyl and the intact-ring oxygens of 171,[18e] steric effects of the bulky substituent at the 6-position of 173 and 178[18b] and at the 8-position of 142 and 149.[18f,116] After oxidation and reduction, their open-chain nucleoside analogs gave the usual positive $[\alpha]_D$ values and/or plain

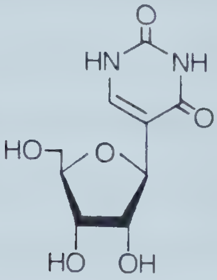
positive curves or positive Cotton effects between 365 and 589 nm. This would imply the existence of increased rotational flexibility around the glycosyl bond of the nucleosides$_{ox\text{-}red}$.

In contrast to these results, 6-azapyrimidine and pyridazine nucleosides$_{ox\text{-}red}$ retain the abnormal (reversed sign) optical rotation behavior of their precursor nucleosides. This suggests that the reversed rotation behavior of 6-azapyrimidine and pyridazine nucleosides arises mainly from the chromophoric effects (intrinsic optical vector orientation in the chromophore) rather than rotational preferences[18e] about the glycosyl bond. Similarly, the reversed chiroptical behavior of 6-azapyrimidine and pyridazine nucleosides$_{ox\text{-}red}$ may be attributed to the same phenomenon.

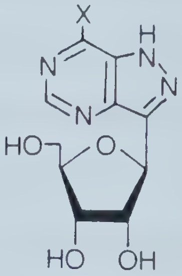
Our results with ox-red products provide additional support for the glycosyl conformational preference rationale for the reversed ORD and CD Cotton effects of the precursor 6-substituted pyrimidine nucleosides (173, 178), 8-substituted purine nucleosides (142, 149) and 3-β-D-ribofuranosyl-6-methyluracil (171). The combined observations suggest that the long wavelength rotation behavior of nucleosides$_{ox\text{-}red}$ may be useful for the study of nucleoside glycosyl rotameric conformational preferences. Comparison of the CD and ORD spectral properties of the precursor nucleosides and the rotations of

the ox-red products with enhanced glycosyl bond flexibility will provide an additional parameter to evaluate steric conformational vs. intrinsic optical vector effects with the base chromophore.

Pseudouridine (5-β-D-ribofuranosyluracil) (194) was known to be labile under the periodate oxidation and borohydride reduction conditions.[88]



194



195; X = NH_2
196; X = OH

An elimination mechanism has been proposed by Chambers.[117] Formycin (195) and formycin B (196) have been reported to give 3-formylpyrazolo[4,3-d]pyrimidine derivatives upon prolonged oxidation with periodate.[89] A similar overoxidation has been reported for a benzimidazole C-nucleoside derivative.[84] Thus, it appears that C-nucleosides can not be subjected directly to the present procedure of periodate oxidation and borohydride reduction. However, this procedure was employed with formycin at the 3'-terminal of a tRNA during a study of the specificity of aminoacyl-tRNA synthetases.[118] The negative results

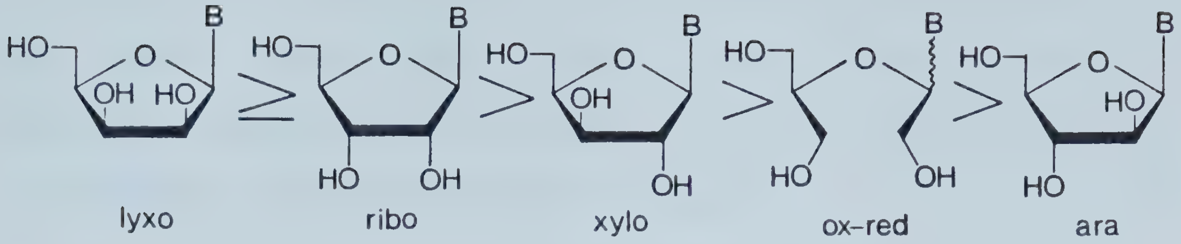
observed in the aminoacylation with the 3'-terminal modified tRNA (formycin$_{\text{ox-red}}$ at 3'-terminal) now would appear to result from degradation of the terminal formycin and not necessarily to the reported specificity effects of the supposed triol product.

In order to circumvent the degradation of these labile C-nucleoside intermediates by the reaction conditions, methylation of the acidic NH functions on the bases was examined. Treatment of pseudouridine (194) with N,N-dimethylformamide dimethyl acetal gave 1,3-dimethylpseudouridine (190) which was subjected to the usual periodate oxidation and borohydride reduction conditions. The resulting 1,3-dimethylpseudouridine$_{\text{ox-red}}$ (*in situ*) gave positive rotation values and a single anodic migrating spot upon electrophoresis. The anodic mobilities of 0.63 for 1,3-dimethylpseudouridine$_{\text{ox-red}}$ (*in situ*) and 0.94 for its precursor 1,3-dimethylpseudouridine indicate that the product is the expected triol (28, B = 1,3-dimethyluracil-5-yl). After oxidation and reduction, synthetic 1-methylformycin (155) and 2-methylformycin (156) gave ox-red products with positive rotations and homogeneous electrophoretic spots corresponding to triol species. The stability of these N-methyl nucleosides toward the reaction conditions was further substantiated by the isolation of 2-methylformycin$_{\text{ox-red}}$ (157). The electrophoretic mobilities and optical rotations of the isolated

and *in situ* prepared 2-methylformycin$_{\text{ox-red}}$ were identical. The stability of the N-methylated formycins (156, 157) and N^1,N^3-dimethylated pseudouridine (190) suggests that the NH group is the key factor in the labilities of pseudouridine and formycin oxidation intermediates. The rotations of these methylated nucleosides$_{\text{ox-red}}$ agree with the usual β (positive) trend.

In most reported cases, no attempt was made to isolate or characterize the ox-red products from nucleosides except for measurement of the optical rotation. The structures of most nucleosides$_{\text{ox-red}}$ (*in situ*) have been presumed on the basis of the quantitative nature of the reactions. Paper electrophoresis was found to be a very useful tool. Electrophoretic examination of nucleosides$_{\text{ox-red}}$ and their intact precursors using a borate buffer at pH 9.3 revealed well separated spots for the two classes and consistent trends were observed in their anodic migrations.

Nucleosides$_{\text{ox-red}}$ migrated faster than arabinofuranosyl nucleosides, but slower than other pentofuranosyl nucleosides (xylo-, lyxo- and ribofuranosyl compounds). The following order of anodic migrations is based on adenine derivatives that were available in the complete set of β-D-pentofuranosyl derivatives. (Scheme VII). This order of migration can be attributed to the ease of formation of ionic, cyclic borate complexes with the



B = adenin-9-yl

Scheme VII

hydroxyl groups.[119] Differences in the electrophoretic migration of anomeric pairs under our conditions was not observed, although the anomeric pair of pseudouridines was reported to separate using a borate buffer at pH 9.6.[120] The electrophoresis of methyl β-D-ribofuranoside$_{ox\text{-}red}$ (*in situ*) was not determined but its structure (as 28, B = OMe) was supported by the correspondence of the reported value of $[\alpha]_D$ +12~15°.[81]

In summary, the previously known trend of β(+) and α(-) rotation signs for nucleosides$_{ox\text{-}red}$ is in accord with most of the nucleosides presently studied. However, 6-azapyrimidine and pyridazine nucleosides are exceptions. This reversed behavior probably results from the intrinsic optical properties of the aglycons. The results suggest that enhanced flexibility exists about the glycosyl bond of nucleosides$_{ox\text{-}red}$. This information can be used in conjunction with ORD/CD studies on the precursor nucleosides for conformational studies of the latter. Formycin and pseudouridine are labile to the

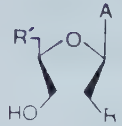
reaction conditions, but their N-methylated derivatives are stable. Paper electrophoresis with a borate buffer at pH 9.3 appears to be reliably useful for qualitative characterization of nucleosides$_{\text{ox-red}}$.

OPTICAL ROTATIONS OF ADENOSINE$_{\text{OX-RED}}$ DERIVATIVES

Lerner[96,97] has employed the empirical trend in the signs of optical rotations of nucleosides$_{\text{ox-red}}$ for assigning the anomeric configuration of adenine nucleosides such as 70 and 82 in Table I. Such compounds give ox-red products with three chiral centers. We have determined the optical rotations of a series of modified adenosine$_{\text{ox-red}}$[121] derivatives that were available in our laboratory. The results are tabulated in Table IV. It can be seen that these modified adenosine$_{\text{ox-red}}$ products (58, 197-208) have positive rotations in agreement with the "normal" β enantiomeric ox-red products. The rationalization for application of the empirical trend to such nucleosides (70, 82)[96,97] is based upon the observation that chiral centers other than the anomeric center do not appear to contribute significantly to the rotation. This is in harmony with ORD and CD studies of pentofuranosyl nucleosides which have shown that the anomeric center is the major contributor to the optical activities.[14,16,18e,20] The configurations of other centers normally are of minor consequence.

TABLE IV

Optical Rotations of a Series of Modified Adenosine$_{ox\text{-}red}$ Derivatives from our Laboratory[121]



Compound	R	R'	$[\alpha]_{365}$	$[\alpha]_{436}$	$[\alpha]_{546}$	$[\alpha]_{578}$	$[\alpha]_{589}$	Concentration and solvent a)
<u>197</u>	OH	TsO	+149.0	+87.0	+49.0	+42.8	+40.4	c 0.53, 20% MeOH
<u>198</u>	TsO	OH	+35.9	+35.6	+25.4	+23.2	+22.2	c 0.39, 20% MeOH
<u>58</u>	OH	H	+188.7	+120.1	+71.1	+59.1	+50.3	c 0.16, H_2O
<u>199</u>	H	OH	+149.8	+98.9	+58.9	+52.1	+49.8	c 0.52, H_2O
<u>200</u>	OH	Cl	+209.6	+132.2	+76.3	+67.4	+64.5	c 0.58, H_2O
<u>201</u>	Cl	OH	+188.4	+124.1	+73.9	+65.5	+62.5	c 0.54, H_2O
<u>202</u>	OH	NH_2	+275.3	+176.8	+104.7	+92.2	+88.5	c 0.49, H_2O
<u>203</u>	NH_2	OH	+176.3	+116.8	+69.7	+61.7	+58.5	c 0.47, H_2O
<u>204</u>	OH	N_3	+37.4	+27.4	+17.5	+15.5	+15.0	c 0.52, H_2O
<u>205</u>	N_3	OH	+256.6	+161.4	+94.4	+83.4	+78.9	c 0.36, H_2O
<u>206</u>	I	OH	+99.4	+75.5	+48.3	+44.7	+41.5	c 0.36, H_2O

continued.....

Table IV (continued)

207	OH	OPO_3H_2	+111.1	+68.3	+42.3	+34.5	+33.4	c 0.28, H_2O
208	Br	OH	+146.8	+100.0	+60.8	+54.0	+51.8	c 0.25, H_2O

a) All optical rotations were measured at room temperature (23~24°C).

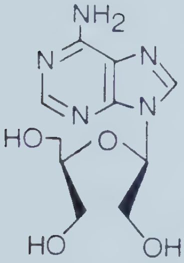
However, a comparison of the optical rotation magnitudes of certain isomer pairs (197 and 198; 204 and 205) undoubtedly reveals the marked influence of the substituent groups. Thus, the contribution from chiral centers other than the anomeric center to the composite optical rotation may be significant in certain cases. Therefore, application of the empirical trend to nucleosides that give ox-red products with more than one chiral center cannot be assumed to provide a secure assignment of the configuration at C-1'. This is especially critical in cases of a small composite rotation magnitude or with structures that have polarizable substituents on chiral centers.

pH AND SOLVENT EFFECTS ON THE OPTICAL ROTATIONS OF ADENOSINE$_{\text{OX-RED}}$ (43), TUBERCIDIN$_{\text{OX-RED}}$ (209), INOSINE$_{\text{OX-RED}}$ (135) AND URIDINE$_{\text{OX-RED}}$ (136)

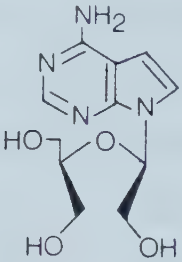
It is well known[122] that solvent modification can effect the chiroptical properties of a compound. Factors to be considered include solute-solvent interactions, dipole-dipole influences, hydrogen bonding, conformational equilibria changes, effects on ionization, etc. Solvent effects have been reported[18b] to cause sign reversals of CD Cotton effects with certain nucleosides. Therefore, the influence of changes in pH and solvents on the optical rotations of two acidic and two basic ionizable nucleo-

sides$_{\text{ox-red}}$ were examined.

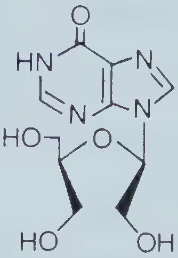
Adenosine$_{\text{ox-red}}$ (43), tubercidin$_{\text{ox-red}}$ (209), inosine$_{\text{ox-red}}$ (135) and uridine$_{\text{ox-red}}$ (136) were synthesized and characterized. The proton NMR spectra of these nucleosides$_{\text{ox-red}}$ had characteristic triplets for the "anomeric" proton signals (J = 6 Hz) due to splitting by the "2'-CH_2OH" group. Solvents examined were MeOH, H_2O, DMF and pyridine. Aqueous (~0.1 M) solutions were measured at pH 1, 3, 5, 7, 9, 11 and 13. Inosine$_{\text{ox-red}}$ was not examined in MeOH because of its insolubility. The rotations were determined at 365, 436, 546, 578 and 589 nm. The results are presented in Tables V, VI, VII and VIII.



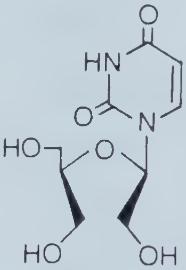
43



209



135



136

In the aqueous solutions, adenosine$_{\text{ox-red}}$ (43), tubercidin$_{\text{ox-red}}$ (209) and inosine$_{\text{ox-red}}$ (135) showed a significant decrease in the rotation magnitude with increased pH. In contrast, uridine$_{\text{ox-red}}$ (136) had a slight increase in magnitude with increased pH, although the rotations at higher wavelengths remained relatively unchanged. All of these nucleosides$_{\text{ox-red}}$ had plain positive

TABLE V

pH and Solvent Effects on the Optical Rotations of Adenosine$_{\text{ox-red}}$ (43)

A. pH effect[a),b)]

	$[\alpha]_{365}$	$[\alpha]_{436}$	$[\alpha]_{546}$	$[\alpha]_{578}$	$[\alpha]_{589}$
pH 1	+266.1	+166.8	+97.1	+85.7	+82.0
2	+236.7	+150.8	+88.4	+77.8	+74.6
5	+221.4	+141.7	+83.0	+73.1	+70.1
7	+226.3	+144.3	+85.2	+75.0	+71.6
9	+217.6	+138.6	+81.4	+71.6	+66.7
11	+213.4	+137.2	+80.4	+71.0	+67.7
13	+161.6	+106.5	+85.7	+55.5	+53.2

B. Solvent effect[a),b)]

	$[\alpha]_{365}$	$[\alpha]_{436}$	$[\alpha]_{546}$	$[\alpha]_{578}$	$[\alpha]_{589}$
MeOH	+228.3	+146.2	+85.6	+75.3	+71.5
H_2O	+226.7	+145.0	+84.9	+74.6	+71.1
pyridine	+55.7	+55.2	+38.4	+34.7	+33.2
DMF	+175.3	+117.2	+70.7	+62.5	+59.9

a) Concentration; 11.16 mg/2 ml. b) Each value obtained is an average of two determinations.

TABLE VI

pH and Solvent Effects on the Optical Rotations of Tubercidin$_{\text{ox-red}}$ (209)

A. pH effect[a),b)]

	$[\alpha]_{365}$	$[\alpha]_{436}$	$[\alpha]_{546}$	$[\alpha]_{578}$	$[\alpha]_{589}$
pH 1	+144.2	+95.6	+57.2	+50.9	+48.4
3	+101.8	+69.8	+42.6	+37.8	+36.0
5	+100.1	+69.0	+44.6	+37.1	+36.0
7	+86.1	+60.5	+37.3	+33.3	+31.9
9	+85.0	+59.0	+37.0	+32.9	+31.3
11	+83.8	+59.1	+36.4	+32.4	+31.2
13	+51.3	+41.0	+26.9	+24.0	+23.1

B. Solvent effect[a),b)]

	$[\alpha]_{365}$	$[\alpha]_{436}$	$[\alpha]_{546}$	$[\alpha]_{578}$	$[\alpha]_{589}$
MeOH	+90.9	+64.4	+40.3	+35.7	+34.2
H_2O	+85.8	+59.8	+37.0	+32.9	+31.9
pyridine	-85.3	-17.5	+0.4	+2.0	+2.3
DMF	+57.9	+51.2	+34.3	+31.0	+29.7

a) Concentration; 13.05 mg/2 ml. b) Each value obtained is an average of two determinations.

[illegible]

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

TABLE VII

pH and Solvent Effects on the Optical Rotations of Inosine$_{ox-red}$ (135)

A. pH effect[a),b)]

	$[\alpha]_{365}$	$[\alpha]_{436}$	$[\alpha]_{546}$	$[\alpha]_{578}$	$[\alpha]_{589}$
pH 1	+275.3	+174.0	+101.6	+89.6	+85.9
3	+221.5	+140.5	+82.3	+72.1	+69.5
5	+224.3	+142.5	+83.6	+73.4	+70.4
7	+224.1	+142.3	+83.2	+73.3	+70.0
9	+218.2	+138.9	+81.6	+71.6	+68.8
11	+197.5	+127.3	+75.2	+66.1	+63.4
13	+154.8	+102.3	+61.1	+54.0	+51.6

B. Solvent effect[a),b)]

	$[\alpha]_{365}$	$[\alpha]_{436}$	$[\alpha]_{546}$	$[\alpha]_{578}$	$[\alpha]_{589}$
H_2O	+223.9	+142.3	+83.5	+73.6	+70.5
pyridine	+16.7	+31.3	+25.2	+22.6	+22.4
DMF	+196.7	+127.8	+75.5	+66.8	+64.0

a) Concentration; 10-14 mg/2 ml. b) Each value obtained is an average of two determinations.

TABLE VIII

pH and Solvent Effects on the Optical Rotations of Uridine$_{ox-red}$ (136)

A. pH effect[a),b)]

	$[\alpha]_{365}$	$[\alpha]_{436}$	$[\alpha]_{546}$	$[\alpha]_{578}$	$[\alpha]_{589}$
pH 1	+112.0	+86.5	+56.4	+50.6	+48.8
3	+113.9	+88.0	+57.4	+51.3	+49.2
5	+114.9	+88.7	+58.1	+52.1	+50.1
7	+110.6	+85.3	+55.7	+50.1	+47.8
9	+115.2	+88.4	+57.5	+51.5	+49.5
11	+124.8	+89.9	+57.2	+51.1	+48.8
13	+138.1	+92.7	+57.3	+49.0	+48.6

B. Solvent effect[a),b)]

	$[\alpha]_{365}$	$[\alpha]_{436}$	$[\alpha]_{546}$	$[\alpha]_{578}$	$[\alpha]_{589}$
MeOH	+153.3	+110.9	+69.7	+62.1	+59.3
H_2O	+117.3	+91.0	+59.3	+53.1	+51.2
pyridine	+211.3	+126.5	+73.2	+64.7	+62.0
DMF	+221.8	+139.2	+82.2	+72.7	+69.9

a) Concentration; 11.21 mg/2 ml. b) Each value obtained is an average of two determinations.

curves between 365 and 589 nm. This suggests that ionization of the chromophoric bases does not drastically affect the long wavelength chiroptical properties of these nucleosides$_{ox-red}$. Thus, these longer wavelength optical rotations are less sensitive to ionization of the chromophore than the ultraviolet-CD measurements, an experimentally useful fact.

In the protic solvents H_2O and MeOH, and the aprotic solvent DMF, these four nucleosides$_{ox-red}$ showed plain positive rotation curves analogous to those obtained in aqueous solutions. This indicates that the hydrogen bonding and dipole-dipole interactions between solvents and nucleosides$_{ox-red}$ exert a minor effect on their rotations at long wavelength (365~589 nm). In pyridine, however, drastic changes in the rotation values were observed with adenosine$_{ox-red}$ (<u>43</u>), inosine$_{ox-red}$ (<u>135</u>) and tubercidin$_{ox-red}$ (<u>209</u>). Tubercidin$_{ox-red}$ in this solvent exhibited a cross over in the sign of rotation between 436 and 546 nm. Uridine$_{ox-red}$ (<u>136</u>) was not affected drastically. It is obvious that pyridine is not functioning merely as a base since parallel changes were not observed at increasing pH values. The normal behavior of uridine$_{ox-red}$ (<u>136</u>) in pyridine suggests that this effect does not result from interactions between solvent and the acyclic side chains. The strong interaction with the purine-type compounds is in harmony with

the known base-stacking[20] (i.e. π-π complex formation with pyridine) effects relative to the weak interactions noted with pyrimidines. Thus, caution must be exercised if chiroptical studies of nucleosides$_{\text{ox-red}}$ are attempted using pyridine or other aromatic "stacking" solvents.

RATES OF PERIODATE REDUCTION BY NUCLEOSIDES

The rate of consumption of periodate by anomeric pairs of D-ribofuranosyl nucleosides (42 and 50; 176 and 177; 161 and 162; 153 and 154), lyxo-A (140), and pentofuranosyl nucleosides having a 2',3'-trans glycol unit (47, 49, and 179) were examined spectrophotometrically by the method of Dixon and Lipkin.[123] The results are summarized in Table IX. Nucleosides having a 2',3'-cis glycol structure were oxidized completely in ~20 min. Nucleosides having a 2',3'-trans diol configuration were oxidized much more slowly. This is consistent with the general rule that cis-diols are more rapidly oxidized than trans-diols.[124] Under the conditions used in this study for preparation of nucleosides$_{\text{ox-red}}$ (*in situ*), ara-A (47), xylo-A (49) and ara-C (179) were oxidized completely in ~4 days (monitored by electrophoresis). However, the concentrations employed in the spectrophotometric assay are much lower resulting in a longer oxidation time owing to the dilution effect. An alternative procedure employing higher concentrations of periodate was noted for compound

TABLE IX

Rates of Periodate Reduction by Nucleosides[e)]

Nucleosides		Nucleoside[a)] added (mole x 10^7)	Periodate added (mole x 10^7)	Wavelength[b)] (nm)	1 min[c)]	1.5 min	2.5 min	5.5 min	10.5 min	15.5 min
adenosine	(42)	0.929	2.468	227	0.859	0.926	0.959	0.968	0.968	0.968
α-adenosine	(50)	0.985	2.468	227	0.696	0.823	0.944	1.025	1.034	1.036
lyxo-A	(140)	1.198	2.468	227	0.614	0.785	0.951	1.032	1.032	1.032
cytidine	(176)	0.985	2.468	227	0.774	0.892	0.986	1.025	1.026	1.026
α-cytidine	(177)	0.611	2.468	227	0.544	0.667	0.845	1.009	1.036	1.037
uridine	(161)	0.970	2.468	230	0.899	1.000	1.055	1.069	1.071	1.071
α-uridine	(162)	1.134	2.468	230	0.572	0.717	0.866	0.972	0.984	0.985
7-β-D-ribofuranosyl-theophylline	(153)	1.015	2.468	230	0.981	1.041	1.054	1.055	1.056	1.056
7-α-D-ribofuranosyl-theophylline	(154)	0.915	2.468	230	0.631	0.794	0.943	1.024	1.027	1.027
ara-A	(47)	9.142	15.896	227	~60% of the periodate per nucleoside was reduced in 9 days					
xylo-A	(49)	7.318	15.896	227	~45% of the periodate per nucleoside was reduced in 9 days					
ara-C	(179)[d)]	3.677	15.896	227	~55% of the periodate per nucleoside was reduced in 6 days					

continued.....

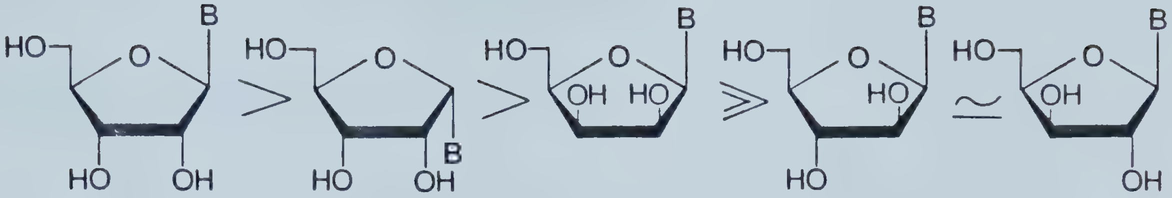
TABLE IX (continued)

a) The concentrations of nucleosides in H_2O were determined using the following extinction coefficients; adenosine and its anomer (ε 15,100 at 259 nm), lyxo-A (ε 14,900 at 259 nm),[125] cytidine (ε 9,100 at 270 nm), α-cytidine (ε 9,100 at 271 nm), uridine (ε 10,300 at 262 nm), α-uridine (ε 10,050 at 264 nm),[126] 7-β-D-ribofuranosyltheophylline (ε 8,700 at 272 nm),[127] 7-α-D-ribofuranosyltheophylline (ε 8,700 at 274 nm), ara-A (ε 13,400 at 259 nm), xylo-A (ε 15,100 at 258 nm),[128] ara-C•HCl salt (ε 9,900 at 273 nm). b) Wavelength at which the decrease in absorbance was followed. c) Each value is an average of three determinations, except two for α-uridine (162). Deviations from the mean values were <±10%. Values are of moles of periodate reduced per mole of nucleoside. d) HCl salt was used. e) Measurements were performed at room temperature (24±2°C).

that are oxidized at inconveniently slow rates.[123]

Comparison of the spectrophotometric rates of periodate reduction clearly demonstrates the possibility of assignment of anomeric configuration of ribofuranosyl nucleosides. During the initial few minutes, ribofuranosyl nucleosides with the β-configuration (42, 176, 161, 153) were oxidized faster than α-anomers (50, 177, 162, 154). The rate of periodate consumption by these β-nucleosides after 1 min is ~1.2 to ~1.6 times faster than for the α-anomers. The formation of a covalently linked, cyclic bidendate complex between the oxidant and a glycol gives the obligatory intermediate.[55,56] Any steric effect that would hinder the formation of this intermediate or inhibit the approach of periodate would reduce the initial rate.[56] Hence, the observed difference in the velocity of periodate consumption by anomeric pairs of ribonucleosides can be explained by the steric hindrance of the α structure. This also accounts for the slower oxidation of lyxo-A (140) relative to α-adenosine (50) since in the former, both 4'-CH_2OH and the chromophore are on the approach-side for the attack of periodate.

The results of the present study give the following order for the oxidation rate (Scheme VIII). However, the rapid oxidation of all nucleosides having a cis 2',3'-diol structure (~20 min. completion) suggests that α and β-anomers may not be distinguishable with higher con-



Scheme VIII

centrations of periodate. These results demonstrate a new method of assigning the anomeric configurations of a given anomeric pair of ribonucleosides using spectrophotometric measurement of the rate of periodate reduction at a concentration of ~10^{-7} $\underline{M}$ IO_4^-.

EXPERIMENTAL

GENERAL PROCEDURES

Melting points were taken on a Reichert microstage apparatus and are uncorrected. A Cary 15 spectrophotometer was used for the determination of UV spectra and reduction rates of periodate by nucleosides. CD and ORD data were obtained on a Jasco ORD-UV-5 (CD ss-20) spectropolarimeter at room temperature (23~25°C) using a 1 cm cell. Optical rotations were taken on a Perkin Elmer Model 141 polarimeter using a 1 dm, 1 ml microcell. Proton NMR spectra were recorded on Varian HA-100 (100 MHz) or Perkin Elmer R32 (90 MHz) instruments in DMSO-d_6 with TMS as an internal standard or D_2O with DOH as standard (δ 4.8 ppm). Electron impact mass spectra were obtained by the mass spectrometry laboratory of this department on an AEI MS-50 instrument using direct probe sample introduction at 150-200°C. Chemical ionization mass spectra were obtained on an AEI MS-12 instrument using ammonia as the ionizing gas. Elemental analyses were determined by the microanalytical laboratory of this department.

Electrophoresis was performed on Whatman No. 1 paper using a Savant flatplate apparatus (HV-3000 A). Evaporations were effected using a Buchler rotary evaporator equipped with a dry-ice condenser under water aspirator

or oil pump vacuum. Hydrogenations were effected using a Parr shaking apparatus. Diffusion crystallization[129] was carried out using alcohol with ether diffusion. Silica gel column chromatography was performed on JT Baker No. 3405 silica gel (60-200 mesh), and alumina column chromatography on Merck Aluminum oxide (acid-washed). Anion and cation exchange chromatography were carried out using Dow chemical Dowex 1x2 (OH^-) and Dowex 50Wx8 (H^+) resins, respectively. Barneby-Cheney AU-4 charcoal for absorption of nucleosides and column chromatography was conditioned prior to use by heating at reflux with 1<u>N</u> HCl for several hours, washing with water to neutrality, refluxing with 1<u>N</u> NaOH for several hours, again washing with water to neutrality, and then washing with methanol, chloroform, and air drying. All solvents used were of reagent grade. Solvents (water, methanol, DMF, pyridine) used for spectrophotometric and polarimetric study were further purified. Water was refluxed over potassium permanganate and distilled twice. Aqueous solutions of specified pH and buffer solutions (pH meter) were prepared using this water. Methanol was refluxed over and distilled from magnesium metal turnings. DMF was redistilled. Pyridine was dried over potassium hydroxide pellets for 18 hours and then distilled. Thin layer chromatography (TLC) was performed on Merck precoated plastic sheets (0.2 mm) or glass plates (0.25 mm, silica gel 60 F-254).

Developed chromatograms were evaluated under UV light (254 or 366 nm) or by spraying with a 5% solution of H_2SO_4 in ethanol and heating the plate to 100-200°C. Solvent systems used for TLC were Solvent S_1: the upper phase of ethyl acetate/*n*-propanol/H_2O (4:1:2), solvent S_2: isopropanol/chloroform/conc. aqueous ammonia (10:10:1), solvent S_3: methanol/chloroform (3:7). Sodium metaperiodate (BDH Chemicals, LTD., England) was used for all periodate oxidations, and sodium borohydride (Fischer Scientific Company, U.S.A.) was used for all reductions of the periodate oxidation products.

NUCLEOSIDE MATERIALS

5-Azacytidine was purchased from Aldrich Chemical Company, Inc. 8-Azaguanosine and 8-azaadenosine were kindly provided by Dr. J.A. Montgomery, Southern Research Institute. α-Adenosine, 7-β-D-ribofuranosyltheophylline, 7-α-D-ribofuranosyltheophylline, α-L-uridine-5'-phosphate dilithium salt, β-L-uridine-5'-phosphate dilithium salt, and α-L-cytidine-5'-phosphate dilithium salt were kindly provided by Dr. B. Shimizu and Dr. M. Kaneko, Sankyo Co., LTD., Japan. Other compounds were available in our laboratory or were synthesized as described (*vide infra*).

PREPARATION OF NUCLEOSIDES$_{\text{OX-RED}}$ (*in situ*), THEIR OPTICAL ROTATION MEASUREMENT AND PAPER ELECTROPHORESIS

Preparation of nucleosides$_{\text{ox-red}}$ (*in situ*)

Method 1. To a nucleoside (≤80 μmol) in a 2 ml volumetric test tube was added a sodium periodate solution (1.2 ml, 1.2-1.5 eq.), and the mixture was stirred magnetically at room temperature while protected from light for 1.5 hr. Sodium borohydride (6-8 mg) was added and the solution was stirred for 30 min. Excess sodium borohydride was destroyed by the slow addition of 1*M* sodium dihydrogen phosphate buffer (pH 6.5) (~0.5 ml), and the solution was stirred for 30 min. The volume was then adjusted to exactly 2 ml by addition of the buffer solution and stirred for an additional 30 min before subjection to the optical rotation measurement. Nucleosides$_{\text{ox-red}}$ (*in situ*) for ORD measurement were prepared in the same manner but eight to ten times less nucleoside was used. A reference solution for the ORD measurement was made following the same procedure with omission of the nucleoside.

Method 2. Ara-A, xylo-A and ara-C (as its HCl salt) were treated in the same manner except the oxidation was continued for 4 days using ~1.5 equivalent of the periodate solution.

Optical rotation measurements

Optical rotations of all nucleosides$_{\text{ox-red}}$ (*in situ*) were measured at room temperature (23-26°C). The

reference solution showed no optical rotation and was used as a base line for the ORD measurement. Specific rotations were calculated based upon the theoretical weight of nucleosides$_{ox\text{-}red}$ produced. Specific rotations were obtained for all nucleosides$_{ox\text{-}red}$ (*in situ*) at the wavelength of the sodium *D* line (589 nm). ORD curves (300-600 nm) and/or specific rotations at wavelengths other than 589 nm (365, 436, 546 and 578 nm bands of mercury) were recorded to examine whether nucleosides$_{ox\text{-}red}$ (*in situ*) gave negative or positive Cotton effects (or plain curves) in these regions.

Paper electrophoresis

Paper electrophoresis of all nucleosides$_{ox\text{-}red}$ (*in situ*) and their precursor nucleosides was performed on Whatman No. 1 paper (25.5 x 56.5 cm) using 0.1*M* sodium borate at pH 9.3, at 1.2-1.5 KV for 1.5 hr. Solutions of the compounds were applied as spots 10 cm from the cathodic end of a paper. Migrations were measured relative to that of uridine as a standard (which migrated ~12 cm toward the anode). Each nucleoside$_{ox\text{-}red}$ (*in situ*) migrated as a single spot. All ox-red products were observed under UV light (254 nm) except 5,6-dihydrouridine$_{ox\text{-}red}$ (*in situ*) and its precursor nucleoside, which were detected by the method of Fink et al,[130] and methyl β-*D*-ribofuranoside$_{ox\text{-}red}$ (*in situ*) and its precursor

glycoside.

A compilation of the above results are summarized in Table III.

pH AND SOLVENT EFFECTS ON OPTICAL ROTATIONS OF ADENOSINE$_{\text{OX-RED}}$ (43), TUBERCIDIN$_{\text{OX-RED}}$ (209), INOSINE$_{\text{OX-RED}}$ (135) AND URIDINE$_{\text{OX-RED}}$ (136)

Aqueous solutions used were: pH 1 (0.1N HCl), pH 3 (0.1M NaH_2PO_4 adjusted with 0.1N HCl), pH 5 (0.1M NaH_2PO_4 adjusted with 0.1N NaOH), pH 7 (0.1M NaH_2PO_4 adjusted with 0.1N NaOH), pH 9 (0.1M Na_2HPO_4 adjusted with 0.1N NaOH), pH 11 (0.1M Na_2HPO_4 adjusted with 0.1N NaOH) and pH 13 (0.1N NaOH adjusted with 1N NaOH).

Water, methanol, N,N-dimethylformamide (DMF), and pyridine were used in the solvent effect study on optical rotations of the title compounds. Since the quantity of crystalline inosine$_{\text{ox-red}}$ (10-14 mg/2 ml) used was not readily soluble in methanol (other than aqueous methanol), only water, DMF and pyridine were used. Each sample was evaluated in the following way: Methanol solutions of adenosine$_{\text{ox-red}}$, tubercidin$_{\text{ox-red}}$ and uridine$_{\text{ox-red}}$ were prepared. Exact aliquots of each solution were pipetted into 2 ml volumetric test tubes and the methanol was evaporated at room temperature. The test tubes containing the residue were dried over P_2O_5 under vacuum at room temperature overnight. The concentration of the aliquot

taken was redetermined by the extinction coefficient of nucleoside$_{ox\text{-}red}$ (adenosine$_{ox\text{-}red}$ ε 15,200 at 260 nm in H_2O, tubercidin$_{ox\text{-}red}$ ε 11,500 at 270 nm in H_2O, and uridine$_{ox\text{-}red}$ ε 10,100 at 262 nm in H_2O). Inosine$_{ox\text{-}red}$ was weighed directly into the 2 ml volumetric test tubes. Each sample was dissolved in 2 ml of the appropriate pH solution or solvent. Optical rotations were measured at room temperature (23-24°C) at 365, 436, 546, 578 and 589 nm. After the measurements, paper electrophoresis was performed to confirm that there was no decomposition or hydrolysis of the compounds in the pH solutions and solvents used.

The results are summarized in Table V-VIII.

RATES OF PERIODATE REDUCTION BY NUCLEOSIDES

The rate of reduction of periodate by nucleosides (adenosine and its α-anomer, lyxo-A, uridine and its α-anomer, cytidine and its α-anomer, 7-β-D-ribofuranosyltheophylline and its α-anomer, ara-A, xylo-A, ara-C) was estimated spectrophotometrically by the method of Dixon and Lipkin.[123]

Method 1. Nucleosides with the readily oxidized 2',3'-cis glycol unit (adenosine and its α-anomer, lyxo-A, uridine and its α-anomer, cytidine and its α-anomer, 7-β-D-ribofuranosyltheophylline and its α-anomer) were followed over a 20 minutes time course.

Four quartz cells (1-cm path length, ~1.5 ml cell capacity) were used. Cell$_1$ was filled with water and used as a blank. A 0.6 ml aliquot of aqueous nucleoside solution (containing ~10^{-7} mole) was added to both cell$_2$ and cell$_4$. Water (0.6 ml) was added to both cell$_2$ and cell$_3$. A 0.6 ml aliquot of 4.113×10^{-4} $\underline{M}$ $NaIO_4/H_2O$ (2.468×10^{-7} mole) was added to cell$_3$. The solutions in cell$_2$ and cell$_3$ were mixed (independently) and their absorbances were recorded. Then, 0.6 ml of the same periodate solution was added to cell$_4$, the contents was mixed rapidly and the absorbance was taken immediately and at regular time intervals (min). An appropriate wavelength was chosen where the absorbance of the nucleoside was minimal and the absorption of periodate anion was close to its maximum value (the periodate anion has a maximum absorption at ~223 nm[131]). When the absorbance of cell$_4$ had declined to a constant value, the oxidation was considered to be complete. Reduction of the equivalents of periodate per nucleoside at a given time was calculated as follows;

$A_{red} = (Acell_2 + Acell_3) - Acell_4$ (at the constant wavelength)

where Ared; the decrease in absorbance by reduction of periodate

$Acell_2$; the absorbance of cell$_2$

$Acell_3$; the absorbance of cell$_3$

$Acell_4$; the absorbance of cell$_4$

$$\frac{\text{Ared}}{\text{Acell}_3 \text{ at zero time}} = \text{the periodate fraction which was reduced}$$

Thus,

$$\frac{\text{Ared}}{\text{Acell}_3 \text{ at zero time}} \times \frac{\text{added periodate (mole)}}{\text{added nucleoside (mole)}} = \begin{array}{l}\text{the ratio of equivalents}\\ \text{of reduced periodate}\\ \text{per nucleoside}\end{array}$$

The absorbance of cell_3 was monitored over a period of 25 min to ascertain any change in absorption of periodate anion due to changes in temperature.[123,131] However, there was but a negligible change.

Method 2. Nucleosides with a slowly oxidized 2',3'-trans glycol unit (xylo-A, ara-A, ara-C) were studied by an analogous method over a period of several days. Aliquots (5 ml) of a nucleoside solution (~10^{-6} mole) were added to 10 ml volumetric flask_1 and flask_3. Water (5 ml) was added to 10 ml volumetric flask_1 and flask_2. A 5 ml portion of periodate solution (~10^{-6} mole) was then added to flask_2 and flask_3. All the solutions were mixed well and protected from light except while rotations were being measured. The absorbances of all the solutions were recorded every 24 hr using water as a blank. The periodate reduction was calculated as in method 1.

The results are summarized in Table IX.

SYNTHESES

Preparation of 6-methylcytidine (178)

This compound was prepared by the general Hibert-Johnson silyl ether method of Niedballa and Vorbrüggen.[132]

6-Methylcytosine[133] (1.25 g, 10 mmole) was silylated by refluxing for 3 hr with hexamethyldisilazane (HMDS) (6 ml) and trimethylchlorosilane (TMCS) (1 ml) with exclusion of moisture. Excess HMDS and TMCS were removed under reduced pressure to leave the silylated 6-methylcytosine as a crystalline solid. A solution of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (4 g, 8 mmole) in dry CH_3CN (60 ml) was added to the silylated 6-methylcytosine, and the mixture was stirred. Then, the mixture was cooled in an ice bath, and stannic chloride (1.3 ml) in dry CH_3CN (8 ml) was added. An additional portion of stannic chloride (1 ml) in dry CH_3CN (8 ml) was added after 1.5 hr. The reaction mixture was stirred at ~25°C for 12 hr and evaporated under reduced pressure at ~25°C. To this residue was added 1,2-dichloroethane (200 ml) and the solution was treated with saturated aqueous sodium bicarbonate solution (300 ml). The resulting emulsion was filtered through a Celite pad. The organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on a column of alumina (18 x 4.5 cm) which was eluted with ethyl acetate/

n-hexane (2:1) (700 ml), followed by ethyl acetate/methanol (9:1) to give 2.93 g of 1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-6-methylcytosine as a solid foam. To 1.12 g of this foam was added methanol (50 ml) saturated with ammonia at 0°C. The solution was sealed in a steel bomb and heated at 60°C overnight. The solution was evaporated to dryness and the residue was partitioned between water (10 ml) and 1,2-dichloroethane (20 ml). The aqueous layer was concentrated to a small volume and applied to a column (21 x 2.5 cm) of Dowex 1x2 (OH^-). The column was eluted with water. The appropriate fractions were collected and evaporated to dryness. The residue was crystallized from methanol/water to give 319 mg of 178. The remainder of the 6-methylcytidine tribenzoate was treated in the same manner to give 725 mg of 178 (overall yield, 1.044 g, 51% from 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose): mp 233-235°C; $[\alpha]_D^{23}$ -47.4° (c 0.18, H_2O) [lit.[133] mp 230-232°C dec., $[\alpha]_D^{30}$ -41.5° (c 1.55, H_2O)]; CD (0.0739 mmole/ℓ, H_2O, room temperature) $[\theta]_{270}$ -0.5×10^3, $[\theta]_{250}$ 1.2×10^3, $[\theta]_{220}$ -13.5×10^3 [lit.[18e] CD (H_2O) $[\theta]_{270}$ -1.2×10^3, $[\theta]_{247}$ 0.8×10^3, $[\theta]_{214}$ -15.0×10^3]; UV $(H_2O)_{max}$ 270 nm (ε 9,600), 233 nm (ε 9,800), $(0.1N\ HCl)_{max}$ 277 nm (ε 14,500), $(0.1N\ NaOH)_{max}$ 272 nm (ε 9,700), 231 nm (ε 9,300); NMR (DMSO-d_6) δ 2.23 (s, 3, 6-CH_3), 3.52 (m, 2, two 5'-H's), 3.72 (m, 1, 4'-H), 4.09 (m, 1, 3'-H), 4.60

(m, 1, 2'-H), 4.85 (m, 2, two OH's), 5.03 (d, 1, J = 6 Hz, OH), 5.47 (d, 1, $J_{1'-2'}$ = 5 Hz, 1'-H), 5.57 (s, 1, 5-H), 7.10 (s, 2, NH_2); MS m/e 240.0747 (M^+-NH_3), calcd for $C_{10}H_{12}N_2O_5$: 240.0746. Anal. Calcd for $C_{10}H_{15}N_3O_5$: C, 46.69; H, 5.88; N, 16.33. Found: C, 46.90; H, 5.99; N, 16.29.

Preparation of 3-β-D-ribofuranosyl-6-methyluracil (171) and 1,3-bis(β-D-ribofuranosyl)-6-methyluracil (172)

An attempt to prepare 6-methyluridine by a reported method[132b] gave only the title compounds.

6-Methyluracil[134] (1.28 g, 10 mmole) was silylated with HMDS (6 ml) and TMCS (1 ml) by refluxing the mixture for 2 hr with exclusion of moisture. After removal of excess HMDS and TMCS, the silylated 6-methyluracil was distilled to obtain 2.5 g of an oil. To this oil in an ice bath was added 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (4 g, 8 mmole) in dry CH_3CN (120 ml) and stannic chloride (1.3 ml) in dry CH_3CN (60 ml) with stirring. The solution was then stirred at 22°C for 4 hr. After work up,[132b] the oily residue was chromatographed on an alumina column (22 x 4.5 cm). Elution was effected with n-hexane/ethyl acetate (9:1) (600 ml), ethyl acetate/n/hexane (2:1) (700 ml) and finally ethyl acetate/methanol (9:1), and 18 ml fractions were collected. Fractions 47-60 were combined and evaporated to give 0.88 g of 1,3-bis-

(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-6-methyluracil as a foam.

This foam was treated with absolute methanol (25 ml) and sodium (0.12 g) was added. The mixture was sealed and kept overnight at room temperature. The resulting solution was evaporated to a small volume under reduced pressure at room temperature. Water (30 ml) was added and the solution was neutralized with Dowex 50W x 8 (H^+) resin. The resin was filtered and the solution was extracted with CH_2Cl_2 (20 ml x 2). The aqueous layer was evaporated to give 295 mg of 172 as a foam (19% from 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose): TLC R_f = 0.18 (solvent S_1), R_f = 0.48 (solvent S_3); UV $(H_2O)_{max}$ 265 nm (ε 11,200), 210 nm (sh) (ε 8,900); ORD (c 0.022, H_2O, room temperature) $[\phi]_{283}$ -2,600, $[\phi]_{258}$ 2,300, $[\phi]_{227}$ -7,700 [lit.[135a] UV $(pH\ 7)_{max}$ 265 nm (log ε 4.07), 2.08 nm (log ε 3.96), ORD (c 0.075) $[\phi]_{283}$ -5,300, $[\phi]_{258}$ 5,100, $[\phi]_{227}$ -12,900]; NMR (DMSO-d_6) δ 2.27 (s, 3, 6-CH_3), 3.30-5.20 (m, 16, sugar protons), 5.46 (d, 1, $J_{1'-2'}$ = 4 Hz, 1'-H of N_1-ribosyl), 5.66 (s, 1, 5-H), 6.02 (d, 1, $J_{1'-2'}$ = 4 Hz, 1'-H of N_3-ribosyl).

Fractions 80-105 were combined and evaporated to give 2.54 g of 3-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-6-methyluracil as a foam. This was debenzoylated using sodium in methanol, as described above (for 172), to give 980 mg of 171 as a foam (48% from 1-O-acetyl-2,3,5-tri-

O-benzoyl-β-D-ribofuranose): TLC $R_f = 0.36$ (solvent S_1), $R_f = 0.61$ (solvent S_3); $[\alpha]_D^{23}$ -26.6° (c 0.12, H_2O); UV $(H_2O)_{max}$ 264 nm (ε 7,600), $(0.1N\ HCl)_{max}$ 264 nm (ε 7,400), $(0.1N\ NaOH)_{max}$ 289 nm (ε 7,900), 224 nm (ε 5,900) [lit.[133] $[\alpha]_D^{27}$ -27.6° (c 1, H_2O), UV (pH 1 and pH 4)$_{max}$ 265 nm (ε 8,700), (pH 11)$_{max}$ 289 nm (ε 9,800), (pH 14)$_{max}$ 289 nm (ε 11,300)]; CD (c 0.0724 mmole/ℓ, H_2O, room temperature) $[\theta]_{270}$ -1.7×10^3, $[\theta]_{244}$ 2.6×10^3 [lit.[18b] $[\theta]_{268}$ -4.3×10^3, $[\theta]_{242}$ 6.2×10^3]; NMR (DMSO-d_6) δ 2.00 (d, 3, J < 1 Hz, 6-CH_3), 3.30-3.80 (m, 3, 4-H and two 5'-H's), 4.08 (t, 1, J = 6 Hz, 3'-H), 4.43 (dd, 1, $J_{1'-2'}$ = 4 Hz, $J_{2'-3'}$ = 6 Hz, 2'-H), 5.37 (d, 1, J < 1 Hz, 5-H), 6.04 (d, 1, $J_{1'-2'}$ = 4 Hz, 1'-H), 11.0 (bs, 1, NH); MS m/e 240.0738 (M^+-H_2O), calcd for $C_{10}H_{12}N_2O_5$: 240.0746.

Preparation of 6-methyluridine (173)

This compound was prepared by the method of Winkley and Robins.[133]

To 6-methylcytidine (220 mg, 0.86 mmole) in 2N acetic acid (35 ml) was added sodium nitrate (593 mg, 8.6 mmole), and the reaction mixture was sealed and stirred at room temperature for 2 days. The solution was neutralized with 1N sodium hydroxide solution and the volume was reduced by evaporation at 35°C. AU-4 charcoal (2.5 g) was added and the mixture was stirred for 4 hr. The charcoal was collected, washed with water, and

extracted for 15 hr with ethanol/conc. aqueous ammonia (9:1) (150 ml) in a soxhlet apparatus. The extract was evaporated to dryness. The residue was dissolved in water and applied to a column (18 x 2.5 cm) of Dowex 50Wx8 (H^+). The column was eluted with water and the appropriate fractions were collected and evaporated. The residue was crystallized from methanol with ether diffusion to give 50 mg (22%) of 173: mp 178-180°C; $[\alpha]_D^{23}$ -45.8° (c 0.07, H_2O) [lit.[133] mp 177-178°C, $[\alpha]_D^{30}$ -28.6° (c 1.52, H_2O), lit.[135a] mp 179-181°C]; CD (c 0.0759 mmole/ℓ, H_2O, room temperature) $[\theta]_{265}$ -0.3×10^3, $[\theta]_{250}$ 0.8×10^3, $[\theta]_{214}$ -9.9×10^3 [lit.[18e] $[\theta]_{265}$ -0.3×10^3, $[\theta]_{250}$ 1.0×10^3, $[\theta]_{214}$ -10.0×10^3]; NMR (DMSO-d_6) δ 2.26 (s, 3, 6-CH_3), 3.40-3.80 (m, 3, 4'-H and two 5'-H's), 4.06 (q, 1, J = 6 Hz, 3'-H), 4.58 (m, 2, 2'-H was overlapped with OH), 4.90 (d, 1, J = 6 Hz, OH), 5.17 (d, 1, J = 6 Hz, OH), 5.44 (d, 1, $J_{1'-2'}$ = 4 Hz, 1'-H), 5.54 (s, 1, 5-H), 11.20 (s, 1, NH); MS m/e 227.0672 (M^+-CH_2OH), calcd for $C_9H_{11}N_2O_5$: 227.0668. Anal. Calcd for $C_{10}H_{14}N_2O_6 \cdot 1/4\ H_2O$; C, 45.72; H, 5.56; N, 10.66. Found: C, 45.87; H, 5.50; N, 10.42.

Preparation of 6-azauridine (182)

The method of Niedballa and Vorbrüggen[132a] gave 1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-6-azauridine in 80% yield. Methanolysis of this 6-azauridine tribenzoate (1.12 g, 2 mmole) using sodium methoxide (6 mmole) in

absolute methanol (30 ml) followed by crystallization of the product from methanol gave 420 mg (85%) of 182: mp 159-160°C; $[\alpha]_D^{23}$ -134.7° (c 0.13, pyridine) [lit.[135b] mp 158°C, $[\alpha]_D^{25}$ -132° (c 0.5, pyridine)]; UV $(H_2O)_{max}$ 262 nm (ε 6,400); CD (c 0.127 mmole/ℓ, H_2O, room temperature) $[\theta]_{260}$ -12.0×10^3 [lit.[18e] $[\theta]_{257}$ -10.0×10^3]; NMR (DMSO-d_6) δ 3.44 (m, 2, two 5'-H's), 3.78 (m, 1, 4'-H), 4.00 (m, 1, 3'-H), 4.20 (m, 1, 2'-H), 4.60 (m, 1, OH), 4.98 (bs, 1, OH), 5.20 (bs, 1, OH), 5.90 (d, 1, $J_{1'-2'}$ = 4 Hz, 1'-H), 7.56 (s, 1, 5-H); MS m/e 246.0723 (M^++1), calcd for $C_8H_{12}N_3O_6$: 246.0726. Anal. Calcd for $C_8H_{11}N_3O_8$: C, 39.19; H, 4.52; N, 17.14. Found: C, 39.06; H, 4.63; N, 17.09.

Preparation of 3-methyl-6-azauridine (184).

A mixture of 6-azauridine (247 mg, 1 mmole), N,N'-dimethylformamide dimethyl acetal (0.96 g, 8 mmole) and dry chloroform (5 ml) was gently refluxed for 7 hr while protected from moisture. The resulting solution was evaporated to a syrup, which was dissolved in water (6 ml) and kept overnight. The aqueous solution was evaporated and the product was purified on a preparative TLC plate (Whatman PLK5F, silica gel, 20 x 20 cm) using solvent system S_1. The main UV absorbing zone (R_f = 0.65) was collected and the product eluted with methanol. Evaporation of the methanol extract and crystallization of the

residue from methanol with ether diffusion gave 209 mg (80%) of 184: mp 125-127°C; $[\alpha]_D^{23}$ -125.6° (c 0.11, pyridine) [lit.[136] mp 124°C, $[\alpha]_D^{25}$ -128° (c 1.3, pyridine)]; UV $(MeOH)_{max}$ 263 nm (ε 6,500); NMR (DMSO-d_6) δ 3.18 (s, 3, CH_3), 3.47 (m, 2, two 5'-H's), 3.82 (q, 1, J = 5 Hz, 4'-H), 4.04 (t, 1, J = 5 Hz, 3'-H), 4.27 (dd, 1, $J_{1'-2'}$ = 3.5 Hz, $J_{2'-3'}$ = 5 Hz, 2'-H), 4.96 (bs, 3, three OH's), 5.97 (d, 1, $J_{1'-2'}$ = 3.5 Hz, 1'-H), 7.66 (s, 1, 5-H); MS m/e 241.0701 (M^+-H_2O), calcd for $C_9H_{11}N_3O_5$: 241.0698. Anal. Calcd for $C_9H_{13}N_3O_6$: C, 41.70; H, 5.06; N, 16.21. Found: C, 41.62; H, 5.14; N, 16.24.

Preparation of 6-azacytidine (183)

6-Azacytidine was prepared by the method described above for 6-methylcytidine (178).

6-Azacytosine[137] (300 mg, 2.7 mmole) was silylated as described in the above preparation of 178. After removal of excess silylating reagents, the product was suspended in dry 1,2-dichloroethane (3 ml) and treated with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (1.21 g, 2.4 mmole) in dry 1,2-dichloroethane (15 ml) in the presence of stannic chloride (0.3 ml), followed by additional stannic chloride (0.3 ml) after 2 hr. The reaction mixture was stirred at room temperature for a total of 20 hr. The reaction mixture was diluted with 1,2-dichloroethane (20 ml) and treated with aqueous saturated sodium

bicarbonate solution (40 ml). The resulting emulsion was filtered through a Celite pad and the organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The residue was applied to an alumina column (15 x 2.5 cm) and the column was eluted with ethyl acetate/ n-hexane (2:1) (200 ml) followed by chloroform/methanol (9:1). Appropriate fractions were collected and evaporated to dryness to give a crystalline solid. This was recrystallized from 95% ethanol to give 1.18 g (88%) of 1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-6-azacytosine (mp 219-220°C). A sample of this 6-azacytidine tribenzoate (550 mg, 0.99 mmole) in methanol (60 ml) saturated with ammonia at 0°C was sealed and kept at room temperature for 3 days. The solvent was evaporated, and the residue was dissolved in water (10 ml). The aqueous solution was extracted with CH_2Cl_2 (10 ml x 3) and evaporated. The residue was crystallized from methanol to give 217 mg (90%) of 183: mp 222-224°C [lit.[138] mp 222-224°C (dec)]; $[\alpha]_D^{23}$ -127.8° (c 0.08, H_2O); UV $(H_2O)_{max}$ 263 nm (ε 7,800), $(0.1N\ HCl)_{max}$ 279 nm (ε 6,800), $(0.1N\ NaOH)_{max}$ 275 nm (ε 5,600), 225 nm (ε 9,300); CD (c 0.1049 mmole/ℓ, H_2O, room temperature) $[\theta]_{270}$ -6.5×10^3; NMR (DMSO-d_6) δ 3.40 (m, 2, two 5'-H's), 3.76 (q, 1, J = 5 Hz, 4'-H), 3.97 (t, 1, J = 5 Hz, 3'-H), 4.20 (t, 1, J = 5 Hz, 2'-H), 4.62 (bs, 1, OH), 4.96 (bs, 2, two OH's), 5.98 (d, 1, $J_{1'-2'}$ = 5 Hz, 1'-H), 7.50 (s, 1, 5-H), 7.90 (bs, 2, NH_2); MS m/e 226.0697 (M^+-H_2O),

calcd for $C_8H_{10}N_4O_4$: 226.0702. Anal. Calcd for $C_8H_{12}N_4O_5$: C, 39.35; H, 4.95; N, 22.94. Found: C, 39.14; H, 4.82; N, 23.24.

Preparation of 6-methyl-2-β-D-ribofuranosyl-3-pyridazone (188)

To a solution of 6-methyl-3-(2H)-pyridazone[144] (300 mg, 2.7 mmole) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (1.36 g, 2.7 mmole) in dry CH_3CN (30 ml) was added stannic chloride (0.4 ml) and the solution was stirred at room temperature for 24 hr with exclusion of moisture. Additional stannic chloride (0.4 ml) was added and the solution was stirred at room temperature for 24 hr. The solution was evaporated at 30°C under reduced pressure. The residue was dissolved in methylene chloride (40 ml) and treated with saturated aqueous sodium bicarbonate solution (40 ml). The resulting emulsion was filtered through a Celite pad. The organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on a silica gel column (18 x 2.5 cm). Elution with ethyl acetate/*n*-hexane (2:1) and crystallization from 95% ethanol gave 700 mg (47%) of 6-methyl-2-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-3-pyridazone: mp 120-121°C; Anal. Calcd for $C_{31}H_{26}N_2O_8$: C, 67.14; H, 4.73; N, 5.05. Found: C, 67.42; H, 4.86; N, 4.97.

The above compound (320 mg, 0.58 mmole) was debenzoylated as described in the above preparation of 6-azacytidine (183). Crystallization of the product from methanol with ether diffusion gave 112 mg (79%) of 188: mp 103-105°C; $[\alpha]_D^{23}$ -105.9° (c 0.09, H_2O); UV $(H_2O)_{max}$ 290 nm (ε 3,500), $(0.1N\ HCl)_{max}$ 290 nm (ε 3,500), $(0.1N\ NaOH)_{max}$ 293 nm (ε 3,500); CD (c 0.1680 mmole/ℓ, H_2O, room temperature) $[\theta]_{288}$ -4.2×10^3, $[\theta]_{236}$ -6.3×10^3, $[\theta]_{216}$ -10.7×10^3; NMR (D_2O) δ 2.43 (s, 3, CH_3), 3.89 (m, two 5'-H's), 4.24 (m, 1, 4'-H), 4.55 (t, 1, $J_{2'-3'}$ = 5.5 Hz, 3'-H), 4.67 (dd, 1, $J_{1'-2'}$ = 3 Hz, $J_{2'-3'}$ = 5.5 Hz, 2'-H), 6.43 (d, 1, $J_{1'-2'}$ = 3 Hz, 1'-H), 7.04 (d, 1, J_{4-5} = 10 Hz, 4-H), 7.49 (d, 1, J_{4-5} = 10 Hz, 5-H); MS m/e 243.0976 $(M^{+}+1)$, calcd for $C_{10}H_{15}N_2O_5$: 243.0980; 242.0897 (M^{+}), calcd. for $C_{10}H_{14}N_2O_5$: 242.0902. Anal. Calcd for $C_{10}H_{14}N_2O_5 \cdot 1/3\ H_2O$: C, 48.39; H, 5.96; N, 11.28. Found: C, 48.42; H, 5.80; N, 11.46.

Preparation of 2-β-D-ribofuranosyl-3-pyridazone (189)

This compound was prepared in an analogous manner to the above preparation of 6-methyl-2-β-D-ribofuranosyl-3-pyridazone (188).

To a solution of 3(2H)-pyridazone[145] (255 mg, 2.7 mmole) and 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (1.31 g, 2.6 mmole) in dry CH_3CN (30 ml) was added stannic chloride (0.4 ml), and the solution was stirred at room temperature

for 24 hr with exclusion of moisture. Additional stannic chloride (0.4 ml) was added and the solution was stirred at room temperature for 48 hr. After work up, the residue was chromatographed on a silica gel column (19 x 2.5 cm). Elution with ethyl acetate/n-hexane (2:1) and crystallization of the product from ethyl acetate gave 165 mg (12%) of 2-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-3-pyridazone: mp 110-112°C (lit.[113] mp 117-118°C); Anal. Calcd for $C_{30}H_{24}N_2O_8$: C, 66.66; H, 4.48; N, 5.18. Found: C, 66.36; H, 4.58; N, 5.08.

The above compound (160 mg, 0.30 mmole) in methanol (30 ml) saturated with ammonia at 0°C was sealed and kept at room temperature for 3 days. After work up, crystallization of the product from methanol with ether diffusion gave 52 mg (77%) of 189: mp 168-170°C; $[\alpha]_D^{23}$ -132.1° (c 0.08, H_2O) [lit.[139] mp 171.5-173.5°C; $[\alpha]_D^{20}$ = -132.3° (c 2.0, H_2O)]; UV $(H_2O)_{max}$ 284 nm (ε 4,000), $(0.1N\ HCl)_{max}$ 285 nm (ε 4,200), $(0.1N\ NaOH)_{max}$ 286 nm (ε 3,900); CD (c 0.1709 mmole/ℓ, H_2O, room temperature) $[\theta]_{283}$ -5.4 x 10^3, $[\theta]_{238}$ -6.3 x 10^3, $[\theta]_{216}$ -10.4 x 10^3 [lit.[113] CD (H_2O) $[\theta]_{290}$ -4.9 x 10^3, $[\theta]_{245}$ -6.2 x 10^3, $[\theta]_{223}$ -10.1 x 10^3]; NMR (D_2O) δ 3.85 (m, 2, two 5'-H's), 4.22 (m, 1, 4'-H), 4.51 (t, 1, $J_{2'-3'}$ = 5.5 Hz, 3'-H), 4.69 (dd, 1, $J_{1'-2'}$ = 3 Hz, $J_{2'-3'}$ = 5.5 Hz, 2'-H), 6.43 (d, 1, $J_{1'-2'}$ = 3 Hz, 1'-H), 7.13 (dd, 1, J_{4-6} = 2 Hz, J_{4-5} = 10 Hz, 4-H), 7.57 (dd, 1, J_{4-5} = 10 Hz, J_{5-6} = 4 Hz, 5-H), 8.15 (dd,

1, J_{4-6} = 2 Hz, J_{5-6} = 4 Hz, 6-H); MS m/e 229.0830 (M^++1), calcd for $C_9H_{13}N_2O_5$: 229.0824; 228.0748 (M^+), calcd for $C_9H_{12}N_2O_5$: 228.0746. Anal. Calcd for $C_9H_{17}N_2O_5$: C, 47.37; H, 5.30; N, 12.28. Found: C, 47.17; H, 5.15; N, 12.17.

Preparation of 1,3-dimethylpseudouridine (190)

A mixture of pseudouridine (73 mg, 0.3 mmole) and *N*,*N*'-dimethylformamide dimethyl acetal (0.42 g, 3.5 mmole) in absolute methanol (5 ml) was refluxed for 24 hr while protected from moisture. The solution was evaporated and the residue was applied to a Dowex 1x2 (OH^-) column (9 x 2.5 cm). The column was eluted with water. Appropriate fractions were collected and evaporated to give a solid. Recrystallization of this product from methanol with ether diffusion gave 45 mg (56%) of 190: mp 177-179°C (lit.[140] 174°C); UV $(MeOH)_{max}$ 270 nm (ε 8,800); NMR (DMSO-d_6) δ 3.17 & δ 3.30 (s & s, 3 & 3, N_1-CH_3 and N_3-CH_3), 3.40-3.80 (m, 3, two 5'-H's and 4'-H), 3.90 (m, 2, 2'-H and 3'-H), 4.51 (d, 1, $J_{1'-2'}$ = 4 Hz, 1'-H), 4.60-5.00 (m, 3, three OH's), 7.78 (s, 1, 6-H); MS m/e 273.1075 (M^++1), calcd for $C_{11}H_{17}N_2O_6$: 273.1084; 272.1006 (M^+), calcd for $C_{11}H_{16}N_2O_6$: 272.1008. Anal. Calcd for $C_{11}H_{16}N_2O_6$: C, 48.53; H, 5.92; N, 10.29. Found: C, 48.52; H, 6.00; N, 10.22.

Preparation of 5,6-dihydrouridine (187)

This compound was prepared by the reported method.[141] Uridine (161) (488 mg, 2 mmole) in water (50 ml) was hydrogenated over 5% Rh-Al_2O_3 (100 mg) at 25 psi for 2 hr. The catalyst was filtered and the aqueous solution was evaporated to give an oil. The oily product was applied to a silica gel column (20 x 2 cm) and the column was eluted with methanol/chloroform (3:7). Fractions (10 ml) 4-16 were combined and evaporated to dryness under reduced pressure to leave a yellow foam. The foam was dissolved in aqueous methanol and decolorized with charcoal. The charcoal was filtered and the solvent was evaporated to give 473 mg (97%) of 187: TLC R_f = 0.38 (solvent S_3); $[\alpha]_D^{23}$ -38.3° (*c* 0.05, H_2O) [lit.[141] $[\alpha]_D^{20}$ -36.8 ± 0.4° (*c* 2.12, H_2O]; NMR (D_2O) δ 2.80 & 3.62 (t & t, 2 & 2, J_{5-6} = 6.5 Hz, 5-CH_2 & 6-CH_2), 3.80 (m, 2, two 5'-H's), δ 4.00-4.44 (m, 3, 2'-H, 3'-H and 4'-H), 5.89 (d, 1, $J_{1'-2'}$ = 6 Hz, 1'-H); MS m/e 228.0746 (M^+-H_2O), calcd for $C_9H_{12}N_2O_5$: 228.0747. Anal. Calcd for $C_9H_{14}N_2O_6 \cdot 1/5$ H_2O: C, 43.27; H, 5.81; N, 11.21. Found: C, 43.37; H, 5.69; N, 11.18.

Preparations of 1-methylformycin (155) and 2-methylformycin (156)

These compounds were prepared by the method of

Townsend et al.[142] Better separation of the two compounds than that reported was effected on a Dowex 1x2 (OH^-) column using stepwise elution with aqueous methanol as noted below.

A mixture of formycin (508 mg, 1.9 mmole) and sodium (56 mg, 2.4 mmole) in dry ethanol (12 ml) was stirred at room temperature for 30 min. Methyl iodide (270 mg, 1.9 mmole) was added to the resulting solution, followed by an additional portion of methyl iodide (270 mg, 1.9 mmole) after 1.5 hr. The solution was stirred overnight at room temperature and evaporated to dryness. The residue was dissolved in water and applied to a column of Dowex 1x2 (OH^-) (20 x 4.5 cm). The column was eluted with water (600 ml), 10% aq. methanol (1200 ml) 30% aq. methanol (1200 ml) and finally 50% aq. methanol, and 6 ml fractions were collected. Fractions 390-468 were combined and evaporated to dryness. The residue was crystallized from methanol with ether diffusion to give 292 mg (55%) of <u>156</u>; TLC R_f = 0.45 (solvent S_2); mp 206-207°C (lit.[142] 205-206°C); $[\alpha]_D^{23}$ -93.0° (<u>c</u> 0.20, MeOH); UV $(H_2O)_{max}$ 317 nm (sh) (ε 8,000), 305 nm (ε 12,200), 295 nm (sh) (ε 10,600), 234 nm (ε 5,600), (0.1<u>N</u> HCl)$_{max}$ 306 nm (ε 10,000), 270 nm (ε 4,600), 260 nm (ε 4,700), 233 nm (ε 9,200), (0.1<u>N</u> NaOH)$_{max}$ 318 (sh) (ε 8,100), 306 nm (ε 12,100), 296 (sh) (ε 10,400), 234 nm (sh) (ε 5,600); NMR (DMSO-d_6) δ 3.58 (m, 2, two 5'-H's), 3.90-4.30 (m, 2,

3'-H and 4'-H), 4.13 (s, 3, N-CH_3), 4.61 (m, 1, 2'-H), 4.99 (d, 1, J = 5 Hz, OH), 5.14 (m, 2, 1'-H was overlapped with OH, $J_{1'-2'}$ = 7.5 Hz), 5.91 (m, 1, OH), 7.58 (s, 2, NH_2), 8.02 (s, 1, 5-H); MS m/e 281.1127 (M^+), calcd for $C_{11}H_{15}N_5O_4$: 281.1124). Anal. Calcd for $C_{11}H_{15}N_5O_4$: C, 46.97; H, 5.38; N, 24.90. Found: C, 46.79; H, 5.41; N, 25.05.

Fractions 553-732 were combined and evaporated to dryness. Crystallization from ethanol-water gave 81 mg (14%) of 155: TLC R_f = 0.25 (solvent S_2); mp 156°C (foaming), 218-219°C (completely melted) (lit.[142] mp 170-173°C foams, dec. > 200°C, lit.[143] mp 96-98°C transition point, dec. > 200°C); $[\alpha]_D^{23}$ -65.6° (c 0.12, MeOH); UV $(H_2O)_{max}$ 315 nm (sh) (ε 6,600), 302 nm (ε 11,000), 294 nm (sh) (ε 10,500), 233 nm (ε 8,600), $(0.1N\ HCl)_{max}$ 302 nm (ε 11,300), 239 nm (ε 10,100), $(0.1N\ NaOH)_{max}$ 314 nm (sh) (ε 8,300), 302 nm (ε 11,500), 294 nm (sh) (ε10,700), 232 nm (ε 8,000); NMR (DMSO-d_6) δ 3.57 (m, 2, two 5'-H's), 3.90-4.20 (m, 2, 3'-H and 4'-H), 4.19 (s, 3, N-CH_3), 4.48 (m, 1, 2'-H), 4.87 (m, 3, 1'-H was overlapped with two OH's, $J_{1'-2'}$ = 7.5 Hz), 5.84 (m, 1, OH), 7.40 (s, 2, NH_2), 8.09 (s, 1, 5-H); MS m/e 281.1118 (M^+), calcd for $C_{11}H_{15}N_5O_4$: 281.1124. Anal. Calcd for $C_{11}H_{15}N_5O_4 \cdot H_2O$: C, 44.15; H, 5.73; N, 23.40. Found: C, 43.91; H, 5.46; N, 23.26.

Preparation of α-L-uridine (163), β-L-uridine (164) and α-L-cytidine (180) from their 5'-phosphate salts

Each nucleoside-5'-phosphate dilithium salt (~10 mg) was dephosphorylated with bacterial alkaline phosphatase (0.1 ml, solution of enzyme supplied by Sigma Chemical Co.) in 0.5M tris(hydroxymethyl)aminomethane buffer (pH 8.0)(1.5 ml) at 37°C for 5 hr. The free nucleoside was purified by descending paper chromatography (Whatman No. 1 paper) using isopropanol/conc. aqueous ammonia/water (7:1:2) as developing solvent. The product was detected by UV light (254 nm); the zone that contained the main UV absorption was cut out (R_f of α-L-uridine 0.30, R_f of β-L-uridine 0.31, R_f of α-L-cytidine 0.29). Each free nucleoside was eluted from the paper strip with water to give α-L-uridine (4.47 mg), β-L-uridine (2.66 mg) and α-L-cytidine (5.95 mg). The above yields were estimated by using the UV extinction values of α-D-uridine[126] (ε 10,050 at 264 nm in H_2O) and uridine[126] (ε 10,300 at 262 nm in H_2O), respectively, and cytidine (ε 9,100 at 270 nm in H_2O).

α-L-uridine: $[\alpha]_D^{24}$ +58.0° (c 0.22, H_2O) [lit.[126] α-D-uridine $[\alpha]_D$ -68° (c 1.0, H_2O)]. β-L-uridine: $[\alpha]_D^{24}$ -8.3° (c 0.13, H_2O) [lit.[126] β-D-uridine $[\alpha]_D$ +4.6° (c 5.2, H_2O)]. α-L-cytidine $[\alpha]_D^{24}$ +94.1° (c 0.30, H_2O).

Preparation of 2-methylformycin$_{\text{ox-red}}$ (157)

To 2-methylformycin (156) (140 mg, 0.5 mmole) was added sodium periodate (128 mg, 0.6 mmole) dissolved in

water (10 ml). The mixture was stirred at room temperature while protected from light for 1.5 hr. Sodium borohydride (40 mg, 1 mmole) was added to the solution and stirring was continued for 30 min at room temperature. Excess sodium borohydride was destroyed with 1M sodium dihydrogen phosphate buffer (pH 6.5) (4 ml), and the solution was stirred for 1 hr. AU-4 charcoal (3 g) was added to the solution and the mixture was stirred for 4 hr. The charcoal was collected by filtration, washed with water and packed into a column (20 x 2.5 cm).

The column was eluted with water (200 ml), followed by 50% aq. CH_3CN. The eluate was evaporated. The residue was dissolved in water and decolorized with charcoal. Evaporation of the water and crystallization of the residue from methanol with ether diffusion gave 103 mg (72%) of 157. Paper electrophoreis of this compound showed an identical anodic mobility (0.45, uridine as a standard) as 2-methylformycin$_{ox\text{-}red}$ (*in situ*): TLC R_f = 0.18 (solvent S_1); mp 140-143°C; specific rotations (*c* 0.50, H_2O) $[\alpha]^{24}_{589}$ +56.8°, $[\alpha]^{24}_{578}$ +59.8°, $[\alpha]^{24}_{546}$ +67.6°, $[\alpha]^{24}_{436}$ +107.4°, $[\alpha]^{24}_{365}$ +130.0°; UV $(H_2O)_{max}$ 317 (sh) (ε 7,900), 305 nm (ε 12,300), 295 nm (sh) (ε 10,800), 234 nm (sh) (ε 6,200), (0.1N HCl)$_{max}$ 306 nm (ε 10,700), 270 nm (ε 5,000), 260 nm (ε 5,200), 233 nm (ε 9,500), (0.1N NaOH)$_{max}$ 318 (sh) (ε 8,000) 306 nm (ε 12,100), 296 nm (sh) (ε 10,500), 234 nm (sh) (ε 6,400); NMR (DMSO-d_6)

δ 3.38 (m, 6, 2'-H_2, 3'-H_2, 5'-H_2), 4.19 (s, 3, N-CH_3), 3.87 (t, 1, J = 5 Hz, 4'-H), 5.16 (t, 1, J = 6 Hz, 1'-H), 7.50 (s, 2, NH_2), 8.04 (s, 1, 5-H); MS m/e 284.1356 (M^++1) , calcd for $C_{11}H_{18}N_5O_4$: 284.1359. _Anal_. Calcd for $C_{11}H_{17}N_5O_4 \cdot 1/3\ H_2O$: C, 45.67; H, 6.16; N, 24.21. Found: C, 45.68; H, 6.07; N, 24.13.

Preparation of 3-methyl-6-azauridine$_{\text{ox-red}}$ (185)

3-Methyl-6-azauridine (184) (100 mg, 0.39 mmole) was treated in the same manner as described above for the preparation of 2-methylformycin$_{\text{ox-red}}$ (157) to obtain 89 mg of 185 as a syrup (85% yield estimated from the extinction coefficient of 3-methyl-6-azauridine (184); ε 6,500 at 263 nm in MeOH). Paper electrophoresis of this compound showed an identical anodic mobility (0.48, uridine as a standard) as 3-methyl-6-azauridine$_{\text{ox-red}}$ (_in situ_); TLC R_f = 0.60 (solvent S_1), R_f = 0.68 (solvent S_3); UV $(MeOH)_{max}$ 263 nm, specific rotations (_c_ 0.52, H_2O) (concentration estimated using the extinction coefficient of 184) $[\alpha]^{23}_{589}$ -26.4°, $[\alpha]^{23}_{578}$ -27.8°, $[\alpha]^{23}_{546}$ -33.6°, $[\alpha]^{23}_{436}$ -73.4°, $[\alpha]^{23}_{365}$ -162.3°; NMR (DMSO-d_6) δ 3.18 (s, 3, CH_3 overlapped with sugar proton), 3.10-3.80 (m, 7, 2'-H_2, 3'-H_2, 5'-H_2 and 4'-H), 4.30-5.10 (bs, 3, three OH's), 5.89 (t, 1, J = 6 Hz, 1'-H), 7.64 (s, 1, 5-H).

Preparation of adenosine$_{\text{ox-red}}$ (43)[108,114,121]

To adenosine (42) (1 g, 3.7 mmole) was added an 80 m*M* of solution of sodium periodate (50 ml, 4 mmole), and the mixture was stirred at room temperature for 2 hr while protected from light. The resulting solution was cooled in an ice bath and sodium borohydride (282 mg, 7.5 mmole) dissolved in water (10 ml) was added. The solution was stirred for 15 min in the ice bath followed by 45 min at room temperature. The solution was neutralized with acetic acid and stirred with AU-4 charcoal (7 g) until UV absorption indicated <5% of the nucleoside remained in solution. The charcoal was collected by filtration and washed with water (~200 ml). The crude product was eluted from the charcoal by continuous extraction for 2 days in a soxhlet apparatus with ethanol/conc. aqueous ammonia (9:1) (300 ml). After evaporation of the extract, the residue was dissolved in water and applied to a column of Dowex 1x2 (OH^-) resin (35 x 2.5 cm). The column was washed with water (150 ml), and the product was eluted with 20% aq. methanol. Appropriate fractions were combined and evaporated. The residue was dissolved in aqueous ethanol and decolorized by treatment with charcoal. Evaporation of the solvent and crystallization of the residue from ethanol with ether diffusion gave 630 mg (63%) of 43: TLC R_f = 0.17 (solvent S_1);

mp 139-141°C; [lit.[121] mp 143-145°C]; UV $(H_2O)_{max}$ 260 nm (ε 15,200), (0.1<u>N</u> HCl)$_{max}$ 258 nm (ε 14,600), (0.1<u>N</u> NaOH)$_{max}$ 261 nm (ε 15,000); NMR (DMSO-d_6) δ 3.18 (m, 2, 3'-H_2), 3.48 (m, 3, 5'-H_2 and 4'-H), 3.86 (t, 2 , J = 6 Hz, 2'-H_2), 4.42 (t, 1, J = 6 Hz, 5'-OH), 4.66 (t, 1, J = 5 Hz, 3'-OH), 5.11 (t, 1, J = 6 Hz, 2'-OH), 5.87 (t, 1, J = 6 Hz, 1'-H), 7.19 (s, 2, NH_2), 8.14 (s, 1, 2-H), 8.25 (s, 1, 8-H); MS m/e 269.1121 (M^+), calcd for $C_{10}H_{15}N_5O_4$: 269.1124. Anal. Calcd for $C_{10}H_{15}N_5O_4$: C, 44.61; H, 5.62; N, 26.01. Found: C, 44.38; 5.45; N, 26.28.

Preparation of tubercidin$_{ox\text{-}red}$ (209)

Tubercidin$_{ox\text{-}red}$ was prepared from tubercidin (158) (1 g, 3.7 mmole) by the same procedure used for the preparation of adenosine$_{ox\text{-}red}$ (43). The product, 580 mg (58%) of 209, was obtained as a hygroscopic foam after co-evaporation with toluene (2 ml x 2), followed by drying at 50°C over P_2O_5 in vacuo overnight: TLC R_f = 0.26 (solvent S_1); UV $(H_2O)_{max}$ 270 nm (ε 11,500), (0.1<u>N</u> HCl)$_{max}$ 272 nm (ε 10,800), (0.1<u>N</u> NaOH)$_{max}$ 270 nm (ε 10,900); NMR (DMSO-d_6) δ 3.00-4.00 (m, 7, 2'-H_2, 3'-H_2, 5'-H_2 and 4'-H), 4.38 (t, 1, J = 6 Hz, OH), 4.57 (t, 1, J = 5 Hz, OH), 4.98 (t, 1, J = 6 Hz, OH), 6.00 (t, 1, J = 6 Hz, 1'-H), 6.56 (d, 1, J = 4 Hz, 5-H), 6.95 (s, 1, NH_2), 7.24 (d, 1, J = 4 Hz, 6-H), 8.04 (s, 1, 2-H); MS (CI/NH_3) 269 (M^++1). Anal. Calcd for $C_{11}H_{16}N_4O_4 \cdot 1/4\ H_2O$: C, 48.44;

H, 6.10; N, 20.54. Found: C, 48.30; H, 6.15; N, 20.65.

Preparation of inosine$_{ox-red}$ (135)[109]

Inosine (160) (1 g, 3.7 mmole) was treated in the same manner as described for the preparation of adenosine$_{ox-red}$ (43). The crude product obtained from evaporation of the extract was dissolved in water and applied to a column of Dowex 1x2 (OH^-) resin (35 x 2.5 cm). The column was washed with water (150 ml), and the product was eluted with a 200 m*M* aqueous solution of triethylammonium bicarbonate (pH 7.5). Evaporation of the eluate, decolorization of the solution with charcoal and crystallization from ethanol (with small amount of water) with ether diffusion gave 743 mg of 135: TLC R_f = 0.11 (solvent S_1); mp 207-209°C; [lit.[109] mp 207°C, $[\alpha]_D^{25}$ +70.2° (*c*, 1.61, H_2O)]; UV $(H_2O)_{max}$ 250 nm (ε 12,500), (0.1*N* HCl)$_{max}$ 249 nm (ε 11,400), (0.1*N* NaOH)$_{max}$ 254 nm (ε 13,000); NMR (DMSO-d_6) δ 3.00-4.00 (m, sugar protons), 3.83 (d, 2, J = 6 Hz, sugar CH_2) 4.40 (bs, 1, OH), 4.70 (bs, 1, OH), 5.10 (bs, 1, OH), 5.84 (t, J = 6 Hz, 1'-H), 8.04 (s, 1, 2-H), 8.20 (s, 1, 8-H); MS m/e 270.0966 (M^+), calcd for $C_{10}H_{14}N_4O_5$: 270.0964. *Anal*. Calcd for $C_{10}H_{14}N_4O_5$: C, 44.44; H, 5.22; N, 20.73. Found: C, 44.51; H, 5.17; N, 20.83.

Preparation of uridine$_{ox-red}$ (136)[108,114]

Uridine (161) (1 g, 4 mmole) was treated in the same manner as described for the preparation of adenosine$_{ox\text{-}red}$ (43). The crude product obtained from evaporation of the extract was dissolved in water and applied to a Dowex 1x2 (OH^-) column (35 x 2.5 cm). The column was washed with water (150 ml), and eluted with a 200 mM aqueous solution of triethylammonium bicarbonate (pH ~ 7.5). Appropriate fractions were collected and evaporated. The residue was dissolved in water, decolorized with charcoal and lyophilized. Further drying over P_2O_5 at 100°C overnight gave 560 mg of 136 as an extremely hygroscopic solid glass: TLC R_f = 0.31 (solvent S_1); UV $(H_2O)_{max}$ 262 nm (ε 10,100), $(0.1N\ HCl)_{max}$ 262 nm (ε 10,100), $(0.1N\ NaOH)_{max}$ 262 nm (ε 7,500); NMR (DMSO-d_6) δ 3.20-3.70 (m, sugar protons), 4.58 (m, 2, two OH's), 5.03 (bs, 1, OH), 5.56 (d, 1, J = 8 Hz, 5-H), 5.77 (t, 1, J = 6 Hz, 1'-H), 7.61 (t, 1, J = 8 Hz, 6-H), 11.14 (bs, 1, NH); MS m/e 215.0671 (M^+-CH_2OH), calcd for $C_8H_{11}N_2O_5$: 215.0668. *Anal*. Calcd for $C_9H_{14}N_2O_6$: C, 43.90; H, 5.73; N, 11.38. Found: C, 43.67; H, 5.88; N, 11.08.

REFERENCES

1. P.A. Levene and W.A. Jacobs, Ber., 42, 2469, 2474 (1909).
2. E.L. Smith, "Vitamin B_{12}", Methuen & Co. Ltd., London, 1965.
3. a) S. Furberg, Acta Chem. Scand., 4, 751 (1950).
   b) S. Furberg, Acta Crst., 3, 325 (1950).
4. M.J. Robins and M. MacCoss, in "Chemistry and Biology of Nucleosides and Nucleotides", R.E. Harman, R.K. Robins and L.B. Townsend, eds., Academic Press, New York, 1973, pp. 311-328.
5. a) B.R. Baker, in "Chemistry and Biology of Purines" A Ciba Foundation Symposium, J & A Churchill LTD., London, 1957, p. 120.
   b) B.R. Baker, J.P. Joseph, R.E. Schaub, and J.H. Williams, J. Org. Chem., 19, 1786 (1954).
6. J.A. Montgomery and K. Hewson, J. Chem. Soc. Chem. Commun., 15 (1969).
7. K.A. Watanabe, D.H. Hollenberg, and J.J. Fox, J. Carbohydr. Nucleosides Nucleotides, 1, 1 (1974).
8. J. Farkaš, L. Kaplan, and J.J. Fox, J. Org. Chem., 29, 1469 (1964).
9. a) C.S. Hudson, J. Am. Chem. Soc., 31, 66 (1909).
   b) C.S. Hudson, Adv. Carbohydr. Chem., 3, 1 (1948).
10. S.J. Angyal, Carbohydr. Res., 77, 37 (1979).

11. a) J.J. Fox and I. Wempen, Adv. Carbohydr. Chem., 14, 283 (1959).

b) J.J. Fox, N.C. Yung, I. Wempen, and M. Hoffer, J. Am. Chem. Soc., 83, 4066 (1961).

12. R.U. Lemieux and M. Hoffer, Can. J. Chem., 39, 110 (1961).

13. T.R. Emerson, Chem. Ind (London)., 2129 (1964).

14. a) T.L.V. Ulbricht, in "Synthetic Procedures in Nucleic Acid Chemistry", Vol. 2, W.W. Zorbach and R.S. Tipson, eds., Wiley-Interscience, New York, 1973, pp. 177-213.

b) A. Holy, G.T. Rogers, and T.L.V. Ulbricht, FEBS Lett., 7, 335 (1970).

c) T.R. Emerson, R.J. Swan, and T.L.V. Ulbricht, Biochemistry, 6, 843 (1967).

d) T.L.V. Ulbricht, T.R. Emerson, and R.J. Swan, Tet. Lett., 1561 (1966).

e) T.R. Emerson, R.J. Swan, and T.L.V. Ulbricht, Biochem. Biophys. Res. Commun., 22, 505 (1966).

f) T.L.V. Ulbricht, T.R. Emerson, and R.J. Swan, Biochem. Biophys. Res. Commun., 19, 643 (1965).

g) T.L.V. Ulbricht, J.P. Jenning, P.M. Scopes, and W. Klyne, Tet. Lett., 695 (1964).

h) G.T. Rogers and T.L.V. Ulbricht, Biochem. Biophys. Res. Commun., 39, 414, 419 (1970).

15. I. Frič, J. Šmejkal, and J. Farkaš, Tet. Lett., 75 (1966).

16. T. Nishimura, B. Shimizu, and I. Iwai, Biochim. Biophys. Acta, 157, 221 (1968).
17. A. Andre and W. Guschlbauer, Nucleic Acid Res., 1, 803 (1972).
18. a) D.W. Miles, S.J. Hahn, R.K. Robins, M.J. Robins, and H. Eyring, J. Phys. Chem., 72, 1483 (1968).
    b) D.W. Miles, M.J. Robins, R.K. Robins, M.W. Winkley, and H. Eyring, J. Am. Chem. Soc., 91, 824, 831 (1969).
    c) D.W. Miles, M.J. Robins, R.K. Robins, and H. Eyring, Proc. Natl. Acad. Sci. USA, 62, 22 (1969).
    d) D.W. Miles, W.H. Inskeep, M.J. Robins, M.W. Winkley, R.K. Robins, and H. Eyring, J. Quantum Chem., III S, 129 (1969).
    e) D.W. Miles, W.H. Inskeep, M.J. Robins, M.W. Winkley, R.K. Robins, and H. Eyring, J. Am. Chem. Soc., 92, 3872 (1970).
    f) D.W. Miles, L.B. Townsend, M.J. Robins, R.K. Robins, W.H. Inskeep, and H. Eyring, J. Am. Chem. Soc., 93, 1600 (1971).
19. J.-F. Chantot and W. Guschlbauer, FEBS Lett., 4, 173 (1969).
20. C.A. Bush, in "Basic Principles in Nucleic Acid Chemistry" vol. II, P.O.P. Ts'o, ed., Academic Press, New York, 1974, pp. 91-169.
21. L.B. Townsend, in "Synthetic Procedure in Nucleic

Acid Chemistry", vol. 2, W.W. Zorbach and R.S. Tipson, eds., Wiley-Interscience, New York, 1973, pp. 267-398.

22. K.L. Rinehardt, Jr., W.S. Chilton, M. Hichens, and W. Von Phillipborn, J. Am. Chem. Soc., 84, 3216 (1962).

23. M. Karplus, J. Chem. Phys., 30, 11 (1959).

24. R.U. Lemieux and D.R. Lineback, Ann. Rev. Biochem., 32, 155 (1963).

25. N. Leonard and R.A. Laursen, J. Am. Chem. Soc., 85, 2026 (1963).

26. M. MacCoss, M.J. Robins, B. Rayner, and J.-L. Imbach, Carbohydr. Res., 59, 575 (1977).

27. C.D. Jardetzky, J. Am. Chem. Soc., 84, 62 (1962).

28. M.J. Robins and M. MacCoss, J. Am. Chem. Soc., 99, 4654 (1977).

29. J.S. Wilson, Ph.D. Dissertation, The University of Alberta, (1981).

30. W.T. Markiewicz, J. Chem. Res., (S), 24 (1979).

31. a) R.J. Cushley, K.A. Watanabe, and J.J. Fox, Chem. Commun., 598 (1966).
    b) R.J. Cushley, K.A. Watanabe, and J.J. Fox, J. Am. Chem. Soc., 89, 394 (1967).
    c) R.J. Cushley, S.R. Lipsky, W.J. McMurray, and J.J. Fox, Chem. Commun., 1611 (1968).

32. a) J.A. Montgomery, Carbohydr. Res., 33, 184 (1974).
    b) J.A. Montgomery and H.J. Thomas, J. Heterocyclic Chem., 16, 353 (1979).

33. D. Horton and J.H. Lauterbach, J. Org. Chem., 34, 86 (1969).

34. a) J.-L. Imbach and B.L. Kam, J. Carbohydr. Nucleosides Nucleotides, 1, 271 (1974).

    b) J.-L. Imbach, J.-L. Barascut, B.L. Kam, B. Rayner, C. Tamby, and C. Tapiero, J. Heterocyclic Chem., 10, 1069 (1973).

    c) J.-L. Imbach, J.-L. Barascut, B.L. Kam, and C. Tapiero, Tet. Lett., 129 (1974).

    d) J.-L. Imbach, Ann. NY Acad. Sci., 255, 177 (1975).

    e) B. Rayner, C. Tapiero, and J.-L. Imbach, Carbohydr. Res., 47, 195 (1976).

35. L.M. Lerner, J. Org. Chem., 44, 4359 (1979).

36. a) P. Fischer, G. Lösch, and R.R. Schmidt, Tet. Lett., 1505 (1978).

    b) R.R. Schmidt, G.R. Lösch, and P. Fischer, Chem. Ber., 113, 2891 (1980).

37. a) G. Mackenzie and G. Shaw, J. Chem. Soc. Chem. Commun., 753 (1977).

    b) G. Mackenzie and G. Shaw, J. Chem. Soc., Perkin I, 1381 (1978).

38. H. Ohrui, G.H. Jones, J.G. Moffatt, M.L. Maddox, A.T. Christensen, and S.K. Byram, J. Am. Chem. Soc., 97, 4602 (1975).

39. E.J. Freyne, J.A. Lepoivre, F.C. Alderweireldt, M.J.O. Anteunis, and A. De Druyn, J. Carbohydr. Nucleosides

Nucleotides, 3, 113 (1976).

40. T. Nishimura and B. Shimizu, Chem. Pharm. Bull. (Tokyo), 13, 803 (1965).

41. C.K. Fay, J.B. Grutzner, L.F. Johnson, S. Sternhell, and P.W. Westerman, J. Org. Chem., 38, 3122 (1973).

42. V.V. Alenin and V.D. Domkin, J. General Chem. USSR, 48, 2388 (1978), translated from Zhurnal Obsche Khimii, 48, 2629 (1978).

43. C.K. Chu, K.A. Watanabe, and J.J. Fox, J. Heterocyclic Chem., 12, 817 (1975).

44. R.J. Cushley, B.L. Blitzer, and S.R. Lipsky, Biochem. Biophys. Res. Commun., 48, 1482 (1972).

45. L.D. Hall and C. Preston, J. Chem. Soc., Chem. Commun., 1319 (1972).

46. S. Tran-Dinh, J.-M. Neuman, J.-M. Thiéry, T. Huyh-Dinh, J. Igolen, and W. Guschlbauer, J. Am. Chem. Soc., 99, 3267 (1977).

47. H. Sugiyama, N. Yamaoka, B. Shimizu, Y. Ishido, and Y. Seto, Bull. Chem. Soc. Jpn., 47, 1815 (1974).

48. a) B.R. Baker and T.E. Walker, Methods in Carbohydrate Chemistry, 8, 151 (1980).
    b) A.J. Gorin and M. Mazurek, Can. J. Chem., 53, 1212 (1975).

49. a) T.J. Cousineau and J.A. Secrist III, J. Org. Chem., 44, 4351 (1979).
    b) R.R. Schmidt and P. Hermentin, Chem. Ber., 112,

2659 (1979).

50. R.S. Wright, G.M. Tener, and H.G. Khorana, J. Am. Chem. Soc., 80, 2004 (1958).

51. C.W. Smith, R.W. Sidwell, R.K. Robins, and R.L. Tolman, J. Med. Chem., 15, 883 (1972).

52. a) V.M. Clark, A.R. Todd, and J. Zussman, J. Chem. Soc., 2952 (1951).
    b) W. Anderson, D.H. Hayes, A.M. Michelson, and A.R. Todd, J. Chem. Soc., 1882 (1954).
    c) A.M. Michelson and A.R. Todd, J. Chem. Soc., 816 (1955).
    d) D.M. Brown, A.R. Todd, and S. Varadarajan, J. Chem. Soc., 868 (1957).

53. R.J. Rousseau, L.B. Townsend, and R.K. Robins, Biochemistry, 5, 756 (1966).

54. a) L. Malaprade, Bull. Soc. Chim. Fr., 39, 325 (1926).
    b) L. Malaprade, Compt. Rend., 186, 382 (1928).

55. A.J. Fatiadi, Synthesis, 229 (1974) and references therein.

56. G. Dryhurst, "Periodate Oxidation of Diol and Other Functional Groups", Pergamon Press, New York, 1970.

57. J.M. Bobbit, Adv. Carbohydr. Chem., 11, 1 (1956).

58. R.D. Guthrie, Adv. Carbohydr. Chem., 16, 105 (1961).

59. R.W. Bailey and J.B. Pridham, Adv. Carbohydr. Chem., 17, 125 (1962).

60. a) G. Schmidt, Methods Enzymol., 12 (part B), 230 (1968).

b) S.H. Leppla, B. Bjoraker, and R.M. Bock, ibid., 236 (1968).

c) J.A. Hunt, ibid., 240 (1968).

d) H.A. Fraenkel-Conrat and A. Steinschneider, ibid., 243 (1968).

61. a) D.M. Brown, H. Fried, and A.R. Todd, Chem. Ind., 352 (1953).

b) P.R. Whitfield, J. Biochem., 58, 390 (1954).

62. a) K. Randerath, E. Randerath, L.S.Y. Chia, R.C. Gupta, and M. Sivarajan, Nucleic Acid Res., 1, 1121 (1974).

b) M. Sivarajan, R.C. Gupta, L.S.Y. Chia, E. Randerath, and K. Randerath, ibid., 1, 1329 (1974).

c) K. Randerath, L.S.Y. Chia, R.C. Gupta, E. Randerath, E.R. Hawkins, C.K. Brum, and S.H. Chang, Biochem. Biophys. Res. Commun., 63, 157 (1975).

63. a) E.L. Jackson and C.S. Hudson, J. Am. Chem. Soc., 59, 994 (1937).

b) W.D. Maclay, R.M. Hann, and C.S. Hudson, J. Am. Chem. Soc., 61, 1660 (1939).

64. B. Lythgoe and A.R. Todd, J. Chem. Soc., 592 (1944).

65. J. Davoll, B. Lythgoe, and A.R. Todd, J. Chem. Soc., 833 (1946).

66. B. Lythgoe, H. Smith, and A.R. Todd, J. Chem. Soc., 355 (1947).

67. D.M. Brown and B. Lythgoe, J. Chem. Soc., 1990 (1950).

68. G.A. Howard, B. Lythgoe, and A.R. Todd, J. Chem. Soc., 1052 (1947).

69. J. Davoll, B. Lythgoe, and A.R. Todd, J. Chem. Soc., 967, 1685 (1948).

70. P. Chang and B. Lythgoe, J. Chem. Soc., 1992 (1950).

71. J.J. Fox, N. Yung, J. Davoll, and G.B. Brown, J. Am. Chem. Soc., 2117 (1956).

72. L.M. Lerner, J. Org. Chem., 34, 101 (1969).

73. N.G. Brink and K. Folkers, J. Am. Chem. Soc., 74, 2856 (1952).

74. a) S. Hanessian and A.R. Pernet, Can. J. Chem., 52, 1266, 1280 (1974).

    b) S. Hanessian, T. Ogawa, and Y. Guidon, Carbohydr., Res., 38, C12 (1974).

75. N. Yamaoka, E. Takahashi, and K. Tuzimura, Carbohydr. Res., 19, 262 (1971).

76. L.M. Lerner, J. Org. Chem., 37, 4386 (1972).

77. J.X. Khym and W.E. Cohn, J. Am. Chem. Soc., 82, 6380 (1960).

78. a) F. Hansske, M. Sprinzl, and F. Cramer, Bioorg. Chem., 3, 367 (1974).

    b) F. Hansske and F. Cramer, Carbohydr. Res., 41, 366 (1975).

    c) F. Hansske and F. Cramer, Carbohydr. Res., 54, 75 (1977).

79. A.S. Jones, A.F. Markham, and R.T. Walker, J. Chem. Soc., Perkin I, 1567 (1976).

80. a) F. Smith and J.W. Van Cleve, J. Am. Chem. Soc., 77, 309 (1955).

b) J.E. Cadotte, C.G.S. Dutton, I.J. Goldstein, B.A. Lewis, F. Smith, and J.W. Van Cleve, J. Am. Chem. Soc., 79, 691 (1957).

c) I.J. Goldstein, B.A. Lewis, and F. Smith, J. Am. Chem. Soc., 80, 939 (1958).

d) T.L. Hullar and F. Smith, J. Org. Chem., 31, 1658 (1966).

81. E. Berner and O. Kjolberg, Acta Chem. Scand., 14, 909 (1960).

82. B.G. Hudson and R. Baker, J. Org. Chem., 32, 2101 (1967).

83. a) M.J. Weiss, J.P. Joseph, H.M. Kissman, A.M. Small, R.E. Schaub, and F.J. McEvoy, J. Am. Chem. Soc., 81, 4050 (1959).

b) F.J. McEvoy, B.R. Baker, and M.J. Weiss, J. Am. Chem. Soc., 82, 209 (1960).

84. C.F. Huebner, R. Lohmar, R.J. Dimler, S. Moore, and K.P. Link, J. Biol. Chem., 159, 503 (1945).

85. H.P.C. Hogenkamp, J.N. Ladd, and H.A. Baker, J. Biol. Chem., 237, 1950 (1962).

86. N.J. Cusack, D.H. Robinson, P.W. Rugg, G. Shaw, and R. Lofthouse, J. Chem. Soc., Perkin I, 73 (1974).

87. J.B. Lee and T.J. Nolan, Tetrahedron, 23, 2789 (1967).

88. a) W.E. Cohn, J. Biol. Chem., 235, 1488 (1960).

b) W.E. Cohn, Biochim. Biophys. Acta, 32, 569 (1959).

89. K. Kawamura, S. Fukatsu, M. Murase, G. Koyama, K. Maeda, and H. Umezawa, J. Antibiotics (Tokyo), 19A, 91 (1966).

90. C.P.J. Claudemans and H.G. Fletcher, Jr., J. Org. Chem., 28, 3004 (1963).

91. P. Kohn and L.M. Lerner, J. Org. Chem., 32, 4076 (1967).

92. L.M. Lerner and P. Kohn, J. Med. Chem., 7, 655 (1964).

93. L.M. Lerner, J. Org. Chem., 40, 2400 (1975).

94. L.M. Lerner and R. Rossi, Biochemistry, 11, 2772 (1972).

95. L.M. Lerner, J. Org. Chem., 43, 161 (1978).

96. L.M. Lerner, J. Org. Chem., 41, 306 (1976).

97. L.M. Lerner, J. Org. Chem., 43, 962 (1978).

98. V.K. Srivastava and L.M. Lerner, Tetrahedron, 34, 2627 (1978).

99. V.K. Srivastava and L.M. Lerner, J. Med. Chem., 22, 24 (1979).

100. E.E. Leutzinger, W.A. Bowles, R.K. Robins, and L.B. Townsend, J. Am. Chem. Soc., 90, 127 (1968).

101. J.A. Montgomery and H.J. Thomas, J. Am. Chem. Soc., 87, 5442 (1965).

102. a) C.L. Schmidt, W.J. Rusho, and L.B. Townsend, Chem. Commun., 1515 (1971).
    b) C.L. Schmidt and L.B. Townsend, J. Org. Chem., 40, 2476 (1975).

103. G.R. Revankar and L.B. Townsend, J. Heterocyclic Chem.,

5, 785 (1968).

104. G.R. Revankar and L.B. Townsend, J. Heterocyclic Chem., 7, 117, 1329 (1970).

105. M. Borek, J. Farkaš, and F. Šorm, Collect. Czech. Chem. Commun., 34, 1690 (1969).

106. M. Borek, J. Farkaš, and F. Šorm, Collect. Czech. Chem. Commun., 32, 3572 (1967).

107. I.A. Korbukh, M.N. Preobrazhenskaya, and O.N. Judia, J. Carbohydr. Nucleosides Nucleotides, 1, 363 (1974).

108. L.M. Lerner, Carbohydr. Res., 13, 465 (1970).

109. R.R. Rossi and L.M. Lerner, J. Med. Chem., 16, 457 (1973).

110. M.J. Robins, M. MacCoss, and G. Ramani, Can. J. Chem., 52, 3803 (1974).

111. a) K. Freudenberg, Stereochemie, Wien, 1932.
    b) C.N. Riiber and N.A. Sørensen, Kgl. Norske Vidensk. Selskab., Skrifter, 1933, No. 7, 50; Chem. Abstr., 28, 4707 (1934).

112. A.S. Cerezo and V. Deulofen, Carbohydr. Res., 2, 35 (1966).

113. Von H. Pischel, P. Nuhn, E. Liedmann, and G. Wagner, J. Prakt. Chemie., 316, 615 (1974).

114. A.M. Kritzyn, S.N. Mikhailov, A. Yu. Misharin, N. Sh. Padyukova, and V.L. Florentiev, Bioorg. Khim., 2, 1338 (1976).

115. J. Donahue and K.N. Trueblood, J. Mol. Biol., 2, 353 (1960).

116. R. Pless, L. Dudycz, R. Stolarski, and D. Shugar, Z. Naturforsch. 33c, 902 (1978).

117. R.W. Chambers, Prog. Nucl. Acid Res. Mol. Biol., 5, 399 (1966).

118. a) A. Maelicke, M. Sprinzl, F. von der Haar, T.A. Khwaja, and F. Cramer, Eur. J. Biochem., 43, 617 (1974).

b) F. von der Haar and E. Gaetner, Proc. Nat. Acad. Sci. USA, 72, 1378 (1975).

c) F. von der Haar and F. Cramer, Biochemistry, 17, 3139 (1978).

119. a) J. Böeseken, Adv. Carbohydr. Chem., 4, 189 (1949).

b) A.B. Foster, Adv. Carbohydr. Chem., 12, 81 (1957).

120. U. Lerch, M.G. Burdon, and J.G. Moffatt, J. Org. Chem., 36, 1507 (1971).

121. G.G. Cross, M.Sc. thesis, The University of Alberta, (1979).

122. P. Crabbé, "ORD and CD in Chemistry and Biochemistry", Academic Press, New York and London, 1972.

123. J.S. Dixon and D. Lipkin, Anal. Chem., 26, 1092 (1954).

124. a) E.F. Garner, I.G. Goldstein, R. Montgomery, and F. Smith, J. Am. Chem. Soc., 80, 1206 (1958).

b) J. Honeyman and C.J.G. Shaw, J. Chem. Soc., 2454 (1959).

c) C.C. Price and H. Kroll, J. Am. Chem. Soc., 60, 2726 (1938).

d) P.E. Fleury, J.E. Courtois, and A. Bieder, Bull. Soc. Chim. Fr., 19, 118 (1952).

e) H. Klosterman and F. Smith, J. Am. Chem. Soc., 74, 5336 (1952).

f) G. Buist, C.A. Bunton, and J.H. Miles, J. Chem. Soc., 743 (1959).

125. E.J. Reist, D.F. Calkins, and L. Goodman, J. Org. Chem., 32, 169 (1967).

126. T. Nishimura, B. Shimizu, and I. Iwai, Chem. Pharm. Bull. (Tokyo), 12, 1471 (1964).

127. K. Onodera, S. Hirano, and F. Masuda, Carbohydr. Res., 4, 263 (1967).

128. M.J. Robins, Y. Fouron, and R. Mengel, J. Org. Chem., 39, 1564 (1974).

129. J.N. Brown and L.M. Trefonas, Organic Preparations and Procedures, 2, 317 (1970).

130. R.M. Fink, R.E. Cline, C. McGanghey, and K. Fink, Anal Chem., 28, 4 (1956).

131. C.E. Crouthamel, A.M. Hayes, and D.S. Martin, J. Am. Chem. Soc., 73, 82 (1951).

132. a) U. Niedballa and H. Vorbrüggen, J. Org. Chem., 39, 3654 (1970).

b) U. Niedballa and H. Vorbrüggen, ibid., 39, 3660 (1970).

133. M.W. Winkley and R.K. Robins, J. Org. Chem., 33, 2822 (1968).

134. J.J. Donleary and M.A. Kise, Org. Syn., 17, 63 (1937).

135. a) M. Prystaš and F. Šorm, Collect. Czech. Chem. Commun., 34, 2316 (1969).
b) M. Prystaš and F. Šorm, ibid., 27, 1578 (1962).

136. M. Prystaš, J. Gut, and F. Šorm, Collect. Czech. Chem. Commun., 27, 1572 (1962).

137. Y. Mizuno, M. Ikehara, and K.A. Watanabe, Chem. Pharm. Bull. (Tokyo), 10, 647 (1962).

138. J. Žemlička and F. Šorm, Collect Czech. Chem. Commun., 30, 2052 (1965).

139. Von H. Pischel and G. Wagner, Arch. Pharm. (Weinheim, Ger.), 300, 737 (1967).

140. K. Hirota, K.A. Watanabe, and J.J. Fox, J. Heterocyclic Chem., 14, 537 (1977).

141. P. Cerutti, Y. Kondo, W.R. Landis, and B. Whitkop, J. Am. Chem. Soc., 90, 771 (1968).

142. L.B. Townsend, R.A. Long, J.P. McGraw, D.W. Miles, and R.K. Robins, J. Org. Chem., 39, 2023 (1974).

143. J. Žemlička, J. Am. Chem. Soc., 97, 5896 (1975).

144. W.G. Overend and L.F. Wiggins, J. Chem. Soc., 239 (1947).

145. R.F. Homer, H. Gregory, W.G. Overend, and L.F. Wiggins, J. Chem. Soc., 2195 (1948).